

AR201-13435B

Robust Summary - Group 10: Fuel Oils

Acute Toxicity

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| <u>Test Substance</u> | Aromatic Pyrolysis Oil, CAS# 64742-90-1. A brown, tarry, highly viscous mixture |
| <u>Method</u> | |
| Method/guideline followed | No guidelines specified; comparable to standard study |
| Type (test type) | Acute limit test |
| GLP | Yes |
| Year | 1982 |
| Species/Strain | Rat, Fischer 344 |
| Sex | Male and female |
| No. of animals per sex per dose | 5 |
| Vehicle | None |
| Route of administration | Oral gavage |
| Test Conditions | Rats (50 days old) were dosed once with undiluted aromatic pyrolysis oil at 5 g/kg. Each rat was observed for clinical signs, mortality and morbidity at 1 and 4 hours after dosing, and daily thereafter for 14 days. Body weight was obtained at initiation and after 7 and 14 days post dosing. At study termination, all rats were sacrificed and gross necropsies performed. |
| <u>Results</u> | |
| LC ₅₀ with confidence limits. | No mortality occurred during the study. Clinical signs included oily staining around the mouth and yellow staining of the inguinal region. No remarkable findings related to test article administration were observed at necropsy. |
| Remarks | |
| <u>Conclusions</u> | Acute oral median lethal dose for aromatic pyrolysis oil was not reached at a dose of 5 g/kg |
| <u>Data Quality</u> | 1. Reliable without restrictions. |
| Reliability | |
| <u>References</u> | Rausina, G.A. 1982. Acute oral toxicity test in albino rats using aromatic pyrolysis oil. Proj. #82-114. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX. |
| <u>Other</u> | |
| Last changed | 7/27/2001 (Prepared by a contractor to the Olefins Panel) |

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Robust Summary - Group 10: Fuel Oils

Acute Toxicity

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| <u>Test Substance</u> | Aromatic Pyrolysis Oil, CAS #64742-90-1. A brown, tarry, highly viscous mixture. |
| <u>Method</u> | No guidelines specified, comparable to standard study. |
| Method/guideline followed | Acute limit test |
| Type (test type) | Yes |
| GLP | 1982 |
| Year | Rat, Fischer 344 |
| Species/Strain | Male and female |
| Sex | 5 |
| No. of animals per sex per dose | Filtered air |
| Vehicle | Whole body inhalation |
| Route of administration | |
| Test Conditions | One group of 10 individually housed rats (5m, 5F; 16-17wks old) was exposed to the aerosolized test substance for 4 hours in stainless steel dynamic exposure chambers, followed by 14 days of post exposure observation for clinical signs, morbidity and death. Body weight was taken before exposure, and 7 and 14 days post exposure. Nominal chamber concentration was 26.8g/m ³ (uncorrected for large particle condensation) and actual concentration was 3.7 g/m ³ as determined by fluorescence. |
| <u>Results</u> | |
| LC ₅₀ with confidence limits. | LC50 was not reached at the dose of 3.7 g/m ³ . |
| Remarks | During exposure, males and females showed excessive tearing and nasal discharge that were absent by day 2 post-exposure. At day 2 and persisting for several days were observations of porphyria around the eyes and ocular discharge. At day 14, the only clinical findings were hair loss and hair discoloration. There were no changes in body weight gain, no deaths, and no abnormal findings at necropsy. |
| <u>Conclusions</u> | LC50 was not reached at 3.7 g/m ³ . |
| <u>Data Quality</u> | |
| Reliability | 1. Reliable without restrictions |
| <u>References</u> | Gordon, T. 1982. LC50 Aromatic Pyrolysis Oil inhalation study in rats. Proj. #82-082. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX |
| <u>Other</u> | |
| Last changed | 7/27/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary - Group 10: Fuel Oils

Genetic Toxicity - in Vitro

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| <p><u>Test Substance</u> <i>Test substance</i></p> <p><u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain</p> <p>Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested</p> <p>Statistical Methods</p> <p>Remarks for Test Conditions</p> | <p>Aromatic Pyrolysis Oil, Gulf. CAS #64742-90-1. Brown-black, highly viscous tarry residue. Composition analysis, purity and stability referred to sponsor.</p> <p>Standard method based on Hsie et al. (1981), O'Neill & Hsie (1979) In vitro mammalian cell forward mutation Chinese hamster ovary (CHO) cell culture Yes 1984 CHO-K-1 heterozygous for hypoxanthine-guanine phosphoribosyl transferase (HGP RT+/-) from Oak Ridge National Laboratory, TN. Yes Rat liver (S9) fraction purchased from Litton Bionetics, Kensington, MD 1.0mg S9 fraction/ml treatment medium (0.3ml S9 fraction in 3 ml medium/flask) Aroclor 1254 induced (treatment not specified) Cytotoxicity: 8, 16, 32, 64, 128, 256, 512, 1023, 2048, 5000µg/ml ± S9; Mutagenicity: 32, 64, 96, 128, 175, 256µg/ml –S9; 128, 175, 256, 375, 512, 750µg/ml +S9; repeat : 500, 600, 750 µg/ml + S9 all diluted in 50% Pluronic[®] polyol F127 (prepared in absolute ethanol, mol. wt. 12,500).</p> <p>Frequency of mutant colonies per million clonable cells was calculated and comparisons of treated cultures with vehicle controls made on transformed data using a two-tailed t-test (Irr & Snee, 1979). Criteria for positive results were significant (p<0.05) increase in mutant colonies (HGPRT+/- ? HGPRT -/-) at any dose level and a dose related response. If only one criterion is met, results are considered equivocal.</p> <p>Sufficient Aromatic Pyrolysis Oil (APO) was weighed separately for each dose level into 10 ml graduated vials; aliquots were kept under an inert nitrogen blanket overnight. 2.5ml of 50% F127 was added to each treatment vial and vehicle control and medium (Ham's F-12 without hypoxanthine) added as required to achieve final 10ml volume for testing. All dosing preparations were thoroughly blended with mixing rods to produce a uniform emulsion, kept in a water bath at 37⁰C until dosing and mixed again just prior to use when 20µl of each preparation was added to 3ml treatment medium/culture vessel. All cultures were incubated at 37⁰C in 5% CO₂ enriched, humidified atmosphere. Positive control mutagens were ethyl methanesulfonate (100µg/ml) for –S9 cultures, and benzo(a)pyrene (4µg/ml) for +S9 cultures. For cytotoxicity, each dose group was composed of 2 flasks, one –S9, one+S9, negative controls ± S9, seeded with 5x10⁵ cells on day 1. Cultures were exposed to test compound for 5 hours on day 2. On day 3, cells were trypsinized and counted with a Coulter Model ZB, then 200 cells were transferred into each of 3 60mm culture dishes. These viability plates were incubated until day 10, fixed in methanol and stained with Giemsa. Colonies were counted visually or with an Artek Model 981 colony counter. Absolute survival = total colony count ÷ number of cells seeded/flask. Relative survival = absolute survival in treated cultures ÷ vehicle control survival. Acceptable survival level is at least 10%. For mutagenicity, cells were seeded on day 1 into 6 flasks/dose group, 3-S9, 3+S9; on day 2 approximately 10⁶ cells were exposed to APO for 5 hours. Vehicle control had 12 flasks, 6-S9, 6+S9. On day 3, cultures with excessive cytotoxicity were discarded. From remaining cultures, 200 cells were seeded to each of 4 viability plates/dose level; incubated to day 10, fixed with methanol, stained with Giemsa, and colonies counted for survival. Expression cultures (10⁵-10⁶ cells/one dish/dose) were seeded on day 3; subcultured three times until day 10 when 200 cells were seeded on each of 4 viability plates/dose and 2x10⁵ cells seeded on each of 5 mutagenicity plates/dose with selective medium containing 10⁻⁵M 6-thioguanine to allow expression of HGPRT mutation. Cultures were incubated undisturbed until day 17-18 when they were fixed and stained. For mutagenicity , a ratio of total colony counts in mutagenicity plates over absolute survival in viability plates was calculated for each treatment group. Frequency of mutant</p> |
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| | colonies/million clonable cells was calculated and statistical comparisons with negative control data were made. |
| <u>Results</u> Genotoxic effects | <p>In the cytotoxicity test, APO induced cell toxicity beginning at 32µg/ml +S9 (cells x10⁵/ml 5.0, 4.3, 3.8, 3.9, 2.5 and 2.3 at 0[vehicle control], 32, 64, 128, 256 and 512µg/ml) and at 256µg/ml –S9 (cells x10⁵/ml 6.2, 6.5, 5.4, 6.4, 0.5 and 2.2 at 0[vehicle], 32, 64, 128, 256 and 512µg/ml). Colony counts after treatment in –S9 cultures (cloned up to 128µg/ml) had no toxicity at any dose level, but +S9 cultures showed significant cytotoxicity at 256µg/ml and higher (relative survival 84.9 and 44.2 at 256 and 512µg/ml). In the mutagenicity test, post treatment reduced cell count was seen at all dose levels ± S9. Addition of S9 to APO treated cultures caused significant toxicity in colony counts at all dose levels (relative survival 64.9, 42.8, 35.4 and 13.6% at 256, 375, 512, 750µg/ml); no significant clonal cytotoxicity seen in –S9 cultures but cloning efficiency was low. (approx. 70 - 75%). Cloning efficiency was impaired at the time of mutant selection in +S9 cultures at 375µg/ml and higher (62.0, 65.9 and 58.6% at 375, 512 and 750µg/ml). A significant increase in mutant frequency compared to vehicle controls was seen at 750µg/ml +S9 cultures accompanied by a somewhat linear dose related response at all dose levels of APO after metabolic activation (mutants/10⁶ clonable cells 10.9, 25.4, 32.2, 14.5 and 49.8 at 0[vehicle] 256, 375, 512 and 750µg/ml). No mutagenic effects were observed in non-activated (-S9) cultures. Positive control compounds demonstrated appropriate responses (EMS-S9 214.5 mutants/10⁶ cells, B(a)P +S9 111 mutants/10⁶ cells). In a repeat trial of the activated portion of the assay, APO induced a significant increase in mutant frequency at 500µg/ml, while higher doses of 600 and 750µg/ml were too toxic to be selected for cloning. A linear dose response was also observed over the clonable dose range (18.0, 30.8, 97.6 and 436.8 mutants/10⁶ cells at 0, 250, 400 and 500µg/ml. These results confirmed the previous positive findings for APO in S9 activated cultures.</p> |
| <u>Conclusions</u> (contracting) | <p>Mutagenic effects of Aromatic Pyrolysis Oil were observed in cultures with metabolic activation at a dose of 750ug/ml (1st trial) and at 500ug/ml (2nd trial) accompanied by linear dose related responses in both trials. Aromatic Pyrolysis Oil induces gene point mutations in the presence of rat liver metabolic activation in CHO/HGPRT cells under conditions of this assay.</p> |
| <u>Data Quality</u> Reliabilities | <p>1. Reliable without restrictions.</p> |
| <u>Reference</u> | <p>Papciak, M.S., Goode, J.W. 1984. CHO/HGPRT test using Aromatic Pyrolysis Oil. Proj. #2081. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX Hsie, A.W. et al. 1981. Mut. Res. 86: 193-214 O'Neill, J.P. and Hsie, A.W. 1979. Banbury Report 2: 55-63 Irr, J.D. and Snee, R.D. 1979. Banbury Report 2: 263-275.</p> |
| <u>Other</u> Last changed | <p>Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel)</p> |

Robust Summary - Group 10: Fuel Oils

Genetic Toxicity - in Vitro

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| <u>Test Substance</u> <i>Test substance</i> | Aromatic Pyrolysis Oil, Gulf. CAS #64742-90-1. brown-black, highly viscous tarry residue. Composition analysis, purity and stability referred to sponsor. |
| <u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested | Standard method based on Williams et al. (1977,1982) In vitro mammalian cell DNA repair assay Unscheduled DNA Synthesis (UDS) in primary hepatocyte cultures. Yes 1984 Fischer 344 male rat (9-10 wks old) – 1 rat per test No NA NA NA Range-finding: 4, 8, 16, 32, 64, 128, 256, 512, 1024 µg/ml: UDS assay 0.5, 2.0, 10, 60µg/ml; all diluted in 50% Pluronic [®] polyol F127 (prepared in absolute ethanol, mol. wt 12,000, 70% hydrophilic) |
| Exposure period Statistical Methods | 18-20 hours None employed. Criteria for positive response are incorporation of radioactive precursor (³ H-thymidine) in cells that are not normally synthesizing DNA, indicating repair of damage. A positive response is defined as a mean net nuclear grain count at any treatment level that exceeds concurrent negative control by at least 6 grains/nucleus; negative control value must not exceed 5 grains. A positive response need not be dose related. |
| Remarks for Test Conditions | Sufficient Aromatic Pyrolysis Oil (APO) was weighed separately for each dose level, 0.75ml of 50% F127 added per ml of final volume and sufficient medium (Williams Medium E with 10% fetal bovine serum and insulin) added to achieve final volume. Test preparations were mixed just prior to addition at 30 or 50µl to each 3 or 5ml culture, respectively. The conc. of ³ H-thymidine (½ life 12.5 yrs.) used in these assays was 1mCi/ml. All cultures were incubated at 37 ⁰ C in 5% CO ₂ enriched humidified atmosphere. For range-finding, primary hepatocytes derived from freshly perfused rat liver were seeded (approx. 1x10 ⁵ cells/ml) into treatment vessels, exposed to test material for 18 hours (2 cultures/dose level; 2 untreated cultures, and two vehicle F127 control cultures), then fixed in formalin and stained with trypan blue for viability determination. At least 50% viability needed for the assay. In the UDS assay, 1x10 ⁵ cells/ml were seeded into coverslip cultures, exposed to ³ H-thymidine and test substance for 18 hours (3 cultures/dose level). Positive control was 2-acetyl aminofluorene (0.2µg/ml). Cells growing on coverslips were rinsed, fixed and glued to microscope slides on day 2. On day 3, slides were dipped in autoradiographic emulsion and stored in the dark at 2-8 ⁰ C. Autoradiographs were developed, stained and coverslipped on day 14. Number of grains overlying 50 randomly selected nuclei/slide were counted. The highest of 3 cytoplasmic grain counts/cell were subtracted to obtain net nuclear grain count. Avg. net nuclear grain count/slide (sum of net nuclear grain count ÷ 50) and mean net nuclear grain count (avg. net nuclear grain count/slide ÷ 3) were calculated. |
| <u>Results</u> Genotoxic effects | APO induced toxicity in primary hepatocytes beginning at 4µg/ml (51.2% viability) following 18-20 hours exposure. Viability continued to decrease in a generally dose related manner to the maximum dose of 1024 µg/ml (0.8% relative viability). UDS occurred in a dose related manner, increasing from 117 net nuclear grains at 2 µg/ml to 218 grains at 60 µg/ml compared to a vehicle control net count of 0.63 and positive control of 363 net nuclear grains. |
| <u>Conclusions</u> (contractor) | Aromatic Pyrolysis Oil induced dose related unscheduled DNA synthesis in cultured rat hepatocytes. Aromatic Pyrolysis Oil causes DNA damage and repair in this assay. |

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| <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p> <p><u>Other</u> <i>Last changed</i></p> | <p>1. Reliable without restrictions. Study conforms to standard design. GLPs have been followed.</p> <p>Brecher, S., Goode, J.W. 1984. Hepatocyte primary culture/DNA repair test of aromatic pyrolysis oil. Proj. #2083. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX</p> <p>Williams, G.M. 1977. Cancer Res. 37: 1845-1851</p> <p>Williams et al. 1977. In Vitro 13: 809-817</p> <p>Williams et al. 1982. Mut. Res. 97:359-370</p> <p>4/11/2001 (Prepared by a contractor to the Olefins Panel)</p> |
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Robust Summary - Group 10: Fuel Oils

Genetic Toxicity - in Vitro

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| <u>Test Substance</u> <i>Test substance</i> | Aromatic Pyrolysis Oil, Gulf. CAS #64742-90-1. Dark brown-black, highly viscous tarry residue. Composition analysis, purity and stability referred to sponsor. |
| <u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested | Standard method based on Cortesi et al (1983), Dunkel et al (1981), Reznikoff et al (1973) In vitro cell transformation Mouse embryo cells Yes 1983 BALB/3T3-A31 -1-1 from T. Kakunaga, National Cancer Inst., 1982 No NA NA NA Cytotoxicity: 4, 8, 16, 32, 64, 128, 256, 512, 1024 µg/ml; Transformation: 8, 16, 32, 64, 128, 256 µg/ml, all diluted in 50% Pluronic [®] polyol F127 (prepared in absolute ethanol, mol. wt. 12,000, 70% hydrophilic). |
| Exposure period Statistical Methods | 2 days None employed. Criteria for positive response were a two-fold increase in type III foci at the highest dose over vehicle control (at least 2 type III foci if vehicle control had none) with or without a dose related response, or a two-fold increase at two or more consecutive doses. Test is equivocal if two-fold increase occurred at any one level other than the highest acceptable dose. |
| Remarks for Test Conditions | Sufficient Aromatic Pyrolysis Oil (APO) was weighed separately for each dose level, 0.75ml of 50% F127 added per ml of final volume and medium (Eagles MEM with 10% heat-inactivated fetal calf serum) added as required to achieve final volume for testing. Test preparations were mixed just prior to addition to cultures at 50µl to each 5 ml culture. All cultures were incubated at 37°C in 5% CO ₂ enriched humidified atmosphere. For cytotoxicity, 2 plate cultures/dose group, 2 plate cultures for vehicle F127 or medium negative control were seeded with 1x10 ⁴ cells/plate in day 1, exposed on days 2-3, trypsinized and counted with a Coulter Model ZB on day 4 for at least 20% survival. For transformation, 15 flasks (1x10 ⁴ cells/flask/dose group) and two plate cultures (100 cells/plate/dose group) were seeded on day 1, exposed on days 2-3 and culture medium changed on day 4. For flask cultures, medium continued to be changed weekly to day 29. Positive control was 3-methylcholanthrene (1µg/ml). Plate cultures were fixed, stained, and counted visually on day 9 to determine cloning efficiency (avg. number colonies/plate ÷ 100 cells seeded). Flask cultures were fixed and stained on day 29 for focus counting and evaluation. Transformation frequency = total type III foci ÷ total flasks/dose group. |
| <u>Results</u> Genotoxic effects | APO induced toxicity in BALB/3T3 cells after two days exposure beginning at 128 µg/ml (59% relative survival); maximum toxicity (80%) occurred between 256-512 µg/ml and plateaued at 1024 µg/ml. APO induced transformed foci at 128, 256 µg/ml, the two highest dose levels, with borderline positives but inconsistent responses at 8-64 µg/ml. Compounds that transform cells have a high probability of inducing tumors if injected into immunosuppressed mice. Positive and negative controls gave expected responses. |
| <u>Conclusions</u> (contractor) | Aromatic Pyrolysis Oil induced transformation in BALB/3T3 cells under conditions of this assay. Cytotoxicity and impairment of cloning efficiency were also observed at the two highest dose levels. |
| <u>Data Quality</u> Reliabilities | 1. Reliable without restriction. |
| <u>Reference</u> | Brecher, S., Good, J.W. 1983. BALB/3T3 transformation test: Aromatic Pyrolysis Oil. Proj. #2084. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil. |

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| <p><u>Other</u> <i>Last changed</i></p> | <p>Pyrolysis Oil. Proj. #2084. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co, Houston, TX Cortesi, E. et al. 1983. Teratogenesis, Carcinogenesis, Mutagenesis 3: 101-110. Dunkel, V.A. et al. 1981. J. Nat'l Cancer Inst. 67: 1303-1315. Reznikoff, C.A. et al. 1973. Cancer Res. 3239-3249.</p> <p>7/27/2001 (Prepared by a contractor to the Olefins Panel).</p> |
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Robust Summary - Group 10: Fuel Oils

Genetic Toxicity - in Vivo

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| <p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period Statistical methods</p> <p>Remarks for Test Conditions.</p> <p><u>Results</u> Genotoxic effects NOAEL (NOEL) LOAEL (LOEL)</p> <p><u>Conclusions</u> (study authors)</p> <p><u>Data Quality</u> Reliabilities</p> <p><u>References</u></p> <p><u>Other</u> Last changed</p> | <p>Aromatic Pyrolysis Oil, Gulf CAS #64742-90-1 Dark brown - black, highly viscous residue. Compositional analysis, purity and stability referred to sponsor.</p> <p>Comparable to standard assay Mammalian bone marrow erythrocyte micronucleus Yes 1984 Mouse CrI:CD[®]-1 (ICR) BR Swiss Male and female: 10M, 10F/group; 15M, 15 F in 1 group (11wks old at initiation) Oral gavage 0.0, 1.25, 2.5, 5.0 g/kg in corn oil 1 dose/day for 2 days; 1 group- 1 dose, 1 day only Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes (PCEs) with micronuclei (MN) , and group mean ratios of PCE to normochromatic erythrocytes (NORMs) were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant ($p<0.05$) increases in micronucleated PCE at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met.</p> <p>Aromatic pyrolysis oil (APO) dosing solutions were prepared fresh for each day of dosing –12.5 g APO mixed with corn oil to make 50 ml, blended by shaking. Three groups of mice were given APO by oral gavage daily for two days. All mice were weighed on day 1 and on day of sacrifice. One half of each treated group and vehicle control (5M, 5F) was killed on day 3 and the remainder on day 4. One group (15M, 15F), given 5.0 g/kg by gavage in a single dose for 1 day only, was killed on days 2, 3, 4 (5/sex/day). Positive control mice given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Slides of femoral bone marrow smears were prepared, stained with May-Grunewald/Giemsa stain and examined microscopically. For each mouse, 1000 PCE and all associated mature erythrocytes (NORMs) were counted. Data collected included group mean body weights for each day, total PCEs, total NORMs, PCEs with MN, and NORMs with MN.</p> <p>Males at all dose levels treated with 1.25-5.0 g/kg APO for 2 days showed statistically significant ($p<0.05$) dose related increases in % micronucleated PCE compared to concurrent negative controls at both day 3 and day 4 sacrifices, except for the 1.25 g/kg group on day 4 which was significantly higher than the historical control only. Females given 2 doses and sacrificed on days 3 and 4 showed statistically significant ($p<0.05$) increases in % micronucleated PCE compared to negative controls at 5.0 g/kg only. All mice given 1 dose of 5.0 g/kg and sacrificed on day 2, 3, 4 showed positive responses compared to negative controls. There were no significant changes in the ratio of PCE/ NORM compared to controls. LOEL (males) = 1.25 g/kg; NOEL (females) = 2.5 g/kg</p> <p>Oral treatment of mice with Aromatic Pyrolysis Oil for 1 or 2 days induced increased frequency of micronucleated polychromatic erythrocytes in bone marrow and is considered positive. APO can induce cytogenetic damage in this test system.</p> <p>1. Reliable without restrictions.</p> <p>Khan, S.H. and Goode, J.W. 1984. Micronucleus test: Aromatic Pyrolysis Oil orally for 2 days. Proj. #2082. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX</p> <p>7/27/2001 (Prepared by a consultant to the Olefins Panel)</p> |
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Robust Summary - Group 10: Fuel Oils

Repeated Dose Toxicity

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| <u>Test Substance</u> | Aromatic Pyrolysis Oil, CAS #64742-90-1. Black tarry material. Refer to sponsor for analysis of composition and purity. |
| Remarks | |
| <u>Method</u> | |
| Method/guideline followed | No guidelines specified, comparable to standard study. |
| Test type | Subacute |
| GLP | Yes |
| Year | 1983 |
| Species | Rat |
| Strain | Fischer 344 |
| Route of administration | Dermal |
| Duration of test | 2 weeks |
| Doses/concentration levels | 0, 1, 2 g/kg |
| Sex | Males and females (5/sex/group), 7 wks old at initiation of study |
| Exposure period | 6 hr./day for 9 days over a 14 day period |
| Frequency of treatment | once/day, 5 days/week |
| Control group and treatment | corn oil, 1.82 ml/kg |
| Post exposure observation period | None |
| Statistical methods | Bartlett's test, Analysis of Variance, Dunnett's test, Kolmogorov-Smirnov two-tailed test |
| Test Conditions | Animals were housed individually in suspended stainless steel cages with wire mesh bottoms and fronts in a room maintained at 69.8 ⁰ F with relative humidity of 46.5% and 12 hour light/dark cycle. Water and chow diet were provided ad lib. Doses of test article were administered over 10% of body surface to the backs of rats clipped free of hair and fitted with Elizabethan collars to reduce ingestion. After 6 hours, collars were removed and residual oil wiped off. Observations for mortality and morbidity were made twice/day on dosing days and once/day on non-dosing days, and for clinical signs at least once daily. Food consumption was determined weekly and body weight at study initiation and weekly thereafter. Dermal reactions were scored on each dosing day, before and after exposure. At sacrifice, gross necropsy was performed and liver, brain, spleen, heart, kidneys and testes were weighed. Slides of sections of the weighed organs, and also ovaries, uterus and skin were prepared for histopathologic examination of control and high dose groups. |
| <u>Results</u> | |
| NOAEL (NOEL) | NOEL not determined. LOEL = 1.0 g/kg |
| LOAEL (LOEL) | No deaths or moribund rats were observed. Food consumption was decreased in all test article dosed rats; in males the decrease was dose related. In both males and females body weight was reduced in a dose related manner. Dermal effects were difficult to evaluate because of the black/tarry test material; however, in the high dose group, after a weekend without dosing, erythema was moderate to severe with fissuring and skin peeling. Skin histopathologic effects consisted of moderate to marked acanthosis and hyperplasia of the epithelium, and hyperkeratosis. No other test article related histopathologic lesions were found. There were no statistically significant changes in clinical chemistry and hematology parameters. Elevated absolute and relative liver weight was observed in all treated groups. |
| Remarks | |
| <u>Conclusions</u> | Aromatic pyrolysis oil at 1.0 and 2.0 g/kg caused depression of body weight gain associated with decreased food consumption. At 2.0 g/kg, all rats showed moderate to severe erythema (Draize score 3-4). Fissures and peeling skin were seen at 2.0 g/kg but not at 1.0 g/kg. The 1.0 g/kg animals were not scored for erythema. Skin changes consisted of hyperplasia and acanthosis. |
| <u>Quality</u> | |
| Reliabilities | 1. Reliable without restrictions |
| <u>References</u> | Zellers, J.E. 1983. Two week repeated dose toxicity study in rats using aromatic pyrolysis oil. Proj. #82-089. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX |
| <u>Other</u> | |
| Last changed | 4/11/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary - Group 10: Fuel Oils

Repeated Dose Toxicity

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| <u>Test Substance</u> | Aromatic Pyrolysis Oil, CAS #64742-90-1. Dark brown to black, highly viscous, tarry residue |
| Remarks | |
| <u>Method</u> | |
| Method/guideline followed | None specified; comparable to standard study. |
| Test type | Subacute |
| GLP | Yes |
| Year | 1983 |
| Species | Rat |
| Strain | Fischer 344 |
| Route of administration | Whole body inhalation |
| Duration of test | 12 days |
| Doses/concentration levels | 0, 0.54, 2.00 g/m ³ (actual) |
| Sex | Male and female (5M, 5F/group) |
| Exposure period | 9 days |
| Frequency of treatment | 6 hr/day |
| Control group and treatment | filtered air, 6 hr/day for 9 days |
| Post exposure observation period | 2 days |
| Statistical methods | Analysis of variance, Dunnett's test, Kolmogorov-Smirnov two tailed test. |
| Test Conditions | Animals (17 wks old) were housed individually and exposed to the test material for 6 hr/day in stainless steel dynamic exposure chambers. Temperature was maintained at 77°F and relative humidity at 45%. Water and chow diet were provided ad lib. Nominal concentrations were measured gravimetrically and actual concentrations by fluorescence. Rats were observed twice daily on dosing days and once daily on non-dosing days for morbidity and mortality, and immediately after exposure for clinical signs. Body weight was taken at initiation, day 5 of treatment, and prior to sacrifice (day 12) after 2 days of recovery. Blood was collected via orbital sinus on day 12 for hematology and clinical chemistry. At sacrifice, necropsies were performed and target organs were weighed and preserved for histopathology. |
| <u>Results</u> | |
| NOAEL (NOEL) | NOEL not determined |
| LOAEL (LOEL) | LOEL = 0.54 g/m ³ |
| Remarks | Males and females showed dose and time related decreases in body weight that were more severe in the male. Males and females showed dose related increases in clinical symptoms (hair loss, nasal discharge, discharge from eyes, eyes closed and perianal soiling). At the high dose, one female showed arching of the back. Clinical pathology values were unremarkable. Treated male and female rats, showed yellow discoloration of the lungs grossly and hyperplasia of the pulmonary alveolar macrophages microscopically. The organ weights of the high dose males' and females' livers, the high dose females' lungs and the low dose females' livers were significantly increased relative to control animal values. The splenic weights of the high dose male and female rats were significantly decreased. A decrease was observed in the high dose male heart weights compared to control male data. |
| <u>Conclusions</u> | No mortality or morbidity was observed. Exposure related signs included weight loss, hair loss, nasal and ocular discharge and arched walking. Skin irritation was observed at both dose levels, which resolved after the 2 days recovery period in the 0.54 g/kg dose group. There were several alterations in absolute and relative organ weights in both sexes at both test article doses. Frequency and severity of effects were related to exposure level. |
| <u>Quality</u> | |
| Reliabilities | 2. Reliable with restrictions. The report is not well written, key parameters that should have been noted in the methods section are scattered in the report. Most effects were clearly dose related but this was not evaluated statistically. |
| <u>References</u> | Gordon, T. 1983. Nine-day repeated dose inhalation toxicity study in rats: Aromatic Pyrolysis Oil. Proj. #2035. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX |
| <u>Other</u> | |
| Last changed | 7/27/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary - Group 10: Fuel Oils

Acute Toxicity

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| <u>Test Substance</u> | Biphenyl Feedstock, CAS #68989-41-3. Dark brown aromatic slurry consisting of crystalline aggregates in a liquid medium. Refer to sponsor for further information. |
| <u>Method</u> | |
| Method/guideline followed | None specified. Comparable to standard study. |
| Type (test type) | Acute |
| GLP | Yes |
| Year | 1983 |
| Species/Strain | Rat, Fischer 344 |
| Sex | Males and females |
| No. of animals per sex per dose | 5M, 5F |
| Vehicle | Corn oil |
| Route of administration | Oral |
| Test Conditions | Rats were individually housed in metal screen-bottomed cages, provided with chow diet and water ad lib and maintained at 72-78°F, with relative humidity of 23-64% and 12 hour light/dark cycle. Doses for the single dose acute LD ₅₀ study were established by performing two preliminary range-finding studies and a more definitive limit test at 5 g/kg. This was followed by testing at 2.5, 3.0, 3.5 and 4.0g/kg. Test article was administered in the form of a 30% (w/v) suspension in corn oil. At study initiation, rats were 91 days old for the limit test and 63 days old for the LD ₅₀ determination. Rats were fasted for 24 hr before dosing, and were observed daily for 14 days after dosing for clinical signs, moribundity and mortality. Gross necropsies were performed on all rats. LD ₅₀ determinations were performed by the method of Litchfield and Wilcoxon. |
| <u>Results</u> | |
| LD ₅₀ with confidence limits. | LD ₅₀ (95% confidence limits), combined sexes = 3.7g/kg (3.3-4.2) LD ₅₀ males = 3.6g/kg (3.08-4.21); LD ₅₀ females = >2.5, <4.0g/kg |
| Remarks | All surviving rats gained weight normally after 7 and 14 days. Deaths/dose are as follows: Males – 2.5g/kg, 0/5; 3.0g/kg, 0/5; 3.5g/kg, 3/5; 4.0g/kg, 4/5; 5g/kg, 5/6. Females – 2.5g/kg, 0/5; 3.0g/kg, 3/5; 3.5 g/kg 0/5; 4.0g/kg, 3/5; 5g/kg, 5/5. Clinical signs included lethargy, excessive lacrimation, dark inguinal stains and diarrhea that persisted until death or diminished in the last several days of the study. At gross necropsy, in rats that died during the study, a black oily substance was usually found in the stomach and/or intestines; this finding was not observed in surviving animals necropsied after 14 days. |
| <u>Conclusions</u> (study author) | Acute oral LD ₅₀ = 3.7g/kg (95% conf. limits 3.3-4.2g/kg). A black oily substance was usually found in the stomach and/or intestines of rats dying during the study. |
| <u>Data Quality</u> | |
| Reliability | 1. Reliable without restriction |
| <u>References</u> | Rausina, G.A. 1983. Acute oral toxicity study in albino rats, Biphenyl Feedstock. Proj. #2036. Gulf Life Sciences Center, Pittsburgh PA for Gulf Oil Chemicals Co., Houston, TX |
| <u>Other</u> Last changed | Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary - Group 10: Fuel Oils

Acute Toxicity

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| <u>Test Substance</u> | Biphenyl Feedstock, CAS #68989-41-3. Amber slurry with an aromatic odor. Refer to sponsor for further information. |
| <u>Method</u> | |
| Method/guideline followed | None specified. Comparable to standard study |
| Type (test type) | Acute limit test |
| GLP | Yes |
| Year | 1983 |
| Species/Strain | Rabbit, New Zealand White |
| Sex | Males and females |
| No. of animals per sex per dose | 5M, 5F |
| Vehicle | None |
| Route of administration | Dermal |
| Test Conditions | Rabbits (3.1 –3.6 kg) were individually housed in suspended metal screen-bottomed cages, in a room maintained at 72-80°F and relative humidity of 34-60%. Neat test material was applied to the shaved abraded skin on backs (4 parallel, lengthwise, epidermal abrasions) at 2g/kg, and the trunk was wrapped with occlusive sheeting. Each rabbit was fitted with an Elizabethan collar to minimize ingestion during the first 24 hrs of exposure, after which time, the collar and wrappings were removed and the test substance wiped off with a dry towel. Observations for mortality and moribundity, clinical signs, and local skin reactions were continued for 14 days. Body wt was recorded prior to testing and at 7 and 14 days. Gross necropsies were performed after sacrifice. |
| <u>Results</u> | |
| LD ₅₀ with confidence limits. | LD ₅₀ was not reached at a single dermal dose of 2g/kg. One treated female rabbit died on day 1 of the study; no clinical signs were observed before the death. Body weights remained stable throughout the study. The other 9 rabbits appeared normal throughout the 14-day observation period. A delayed skin reaction was observed on day 5 when a well-defined skin irritation was noted in all test rabbits. This irritation decreased during the remainder of the study. Desquamation of skin was found after day 8 in 8 of 9 rabbits. Gross necropsies on all rabbits showed no findings attributable to test article administration. |
| Remarks | |
| <u>Conclusions</u> (study author) | LD ₅₀ was not reached at a single dose of 2g/kg. The test article produced a delayed skin irritation that first appeared at day 5 and resolved by day 12. |
| <u>Data Quality</u> | |
| Reliability | 1. Reliable without restrictions |
| <u>References</u> | Rausina, G.A. 1983. Acute dermal toxicity study in albino rabbits with Biphenyl feedstock. Proj. #2037. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX |
| <u>Other</u> | |
| Last changed | Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary - Group 10: Fuel Oils

Acute Toxicity

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| <u>Test Substance</u> | Biphenyl Feedstock, CAS #68989-41-3. Light brown and waxy as solid; amber color as liquid at 70°C. Refer to sponsor for further information. |
| <u>Method</u> | |
| Method/guideline followed | None specified. Comparable to standard study. |
| Type (test type) | Acute limit test |
| GLP | Yes |
| Year | 1982 |
| Species/Strain | Rat, Fischer 344 |
| Sex | Males and females |
| No. of animals per sex per dose | 10 rats (5M, 5F)/group |
| Vehicle | filtered air |
| Route of administration | Inhalation |
| Test Conditions | One group of Fischer 344 rats was exposed to aerosolized test article at a concentration of 3.0g/m ³ (actual) for 4 hr on day 1, followed by 13 days of post-exposure observation for mortality, moribundity, and clinical signs of toxicity. Rats were sacrificed on day 14. Food and water were available ad lib. Rats weighed between 152g and 262g at initiation. Body wt was recorded prior to exposure and on days 7 and 14 (prior to sacrifice and necropsy). The test substance was aerosolized with a nebulizer. Melted test article (80°C) was poured into the nebulizer maintained at approx. 75°C during atmosphere generation. A condensing flask prevented large diameter particles from entering the exposure chamber. Actual chamber concentration was 3.0g/m ³ (TWA) that was primarily particulate with mass median aerodynamic diameter of 4.3 microns. |
| <u>Results</u> | |
| LC ₅₀ with confidence limits. | LC ₅₀ was not reached at 3.0g/m ³ . No deaths occurred from the single 4 hr exposure to 3.0g/m ³ , which was the maximum attainable concentration of test article. Immediately after exposure, all rats were covered with crystalline test article; nearly every rat had dry red material around nose and mouth, perianal soiling, clear ocular discharge, porphyrin around the eyes, and discolored fur. Two males and one female showed labored respiration. These symptoms subsided during the observation period, and at sacrifice, only discolored fur was seen. Body wt was unchanged. No test article related lesions were detected at necropsy. |
| Remarks | |
| <u>Conclusions</u> (study author) | LC ₅₀ was not reached at 3.0g/m ³ and no evidence of systemic toxicity was observed. |
| <u>Data Quality</u> | |
| Reliability | 1. Reliable without restrictions |
| <u>References</u> | Gordan, T. 1982. Acute LC ₅₀ inhalation toxicity test in rats with Biphenyl feedstock. Study # 82-086. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX |
| <u>Other</u> | |
| Last changed | 6/07/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary - Group 10: Fuel Oils

Genetic Toxicity - in Vitro

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| <u>Test Substance</u> <i>Test substance</i> | Biphenyl Feedstock, CAS #68989-41-3. Amber-colored liquid with aromatic odor. Compositional analysis, purity and stability referred to sponsor. |
| <u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested | Standard method based on Hsie et al. (1981), O'Neill & Hsie (1979) In vitro mammalian cell forward mutation Chinese hamster ovary (CHO) cell culture Yes 1984 CHO-K-1 heterozygous for hypoxanthine-guanine phosphoribosyl transferase (HGPRT+/-) from Oak Ridge National Laboratory, TN. Yes Rat liver (S9) fraction purchased from Litton Bionetics, Kensington, MD 1.0mg S9 fraction/ml treatment medium Aroclor 1254 induced (treatment not specified) Cytotoxicity: 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048µg/ml ± S9; Mutagenicity: 4, 8, 16, 21, 26, 32, 64µg/ml ±S9; all diluted in 10% Pluronic® polyol F68 (prepared in deionized water, mol. wt. 8350). |
| Statistical Methods | Frequency of mutant colonies per million clonable cells, corrected for absolute survival by viability plates, was calculated and comparisons of treated cultures with vehicle controls made on transformed data using a two-tailed t-test (Irr & Snee, 1979) using the MUTANT computer program (Snee et al., 1981). Criteria for positive results were a significant (p<0.05) increase in mutant colonies (HGPRT+/- ? HGPRT-/-) at any dose level and a dose related response. If only one criterion is met, results are considered equivocal. |
| Remarks for Test Conditions | Sufficient Biphenyl feedstock was weighed separately for each dose level into 10ml volumetric flasks and stored overnight. The following day, flasks were placed in a water bath heated to approx. 93°C to melt the biphenyl feedstock, then 7.0ml of 10% F68 was added to the highest dose flask, and to each subsequent dose preparation and vehicle control flask along with sufficient medium (Ham's F-12 without hypoxanthine) to achieve final 10ml volume for testing. All flasks were replaced in the water bath. Dosing preparations were vortexed after addition of all components, and just prior to dosing when 20µl were added to each 3 ml culture. All cultures were incubated at 37°C in 5% CO ₂ enriched, humidified atmosphere. Positive control mutagens were ethyl methanesulfonate (100µg/ml) for -S9 cultures, and benzo(a)pyrene (4µg/ml) for +S9 cultures. For range finding (cytotoxicity), each dose group was composed of 2 flasks, one -S9, one+S9, negative controls ± S9, seeded with 5x10 ⁵ cells on day 1. Cultures were exposed to test compound for 5 hours on day 2. On day 3, cells were trypsinized and counted with a Coulter Model ZB, then 200 cells were transferred into each of 3 60mm culture dishes. These viability plates were incubated until day 10, fixed in methanol and stained with Giemsa. Colonies were counted visually or with an Artek Model 981 colony counter. Absolute survival = total colony count ÷ number of cells seeded/flask. Relative survival = absolute survival in treated cultures ÷ vehicle control survival. Acceptable survival level is at least 10%. For mutagenicity, cells were seeded on day 1 into 6 flasks/dose group, 3-S9, 3+S9; on day 2 approximately 10 ⁶ cells were exposed to biphenyl feedstock for 5 hours. Vehicle control had 12 flasks, 6-S9, 6+S9. On day 3, cultures with excessive cytotoxicity were discarded. From remaining cultures, 200 cells were seeded to each of 4 viability plates/dose level; incubated to day 10, fixed with methanol, stained with Giemsa, and colonies counted for survival. Expression cultures (10 ⁵ -10 ⁶ cells/one dish/dose) were seeded on day 3; subcultured three times until day 10 when 200 cells were seeded on each of 4 viability plates/dose and 2x10 ⁵ cells seeded on each of 5 mutagenicity plates/dose with selective medium containing 10 ⁻⁵ M 6-thioguanine to allow expression of HGPRT mutation. Cultures were incubated undisturbed until day 17 when they were fixed and stained. For mutagenicity, a ratio of total colony counts in mutagenicity plates over absolute survival in |

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| <p><u>Results</u> Genotoxic effects</p> <p><u>Conclusions</u> (contractor)</p> <p><u>Data Quality</u> Reliabilities</p> <p><u>Reference</u></p> <p><u>Other</u> Last changed</p> | <p>viability plates was calculated for each treatment group. Frequency of mutant colonies/million clonable cells was calculated and statistical comparisons with negative control data were made.</p> <p>In the cytotoxicity test, Biphenyl feedstock induced toxic effects at concentrations of 32 µg/ml and higher –S9, and at 16µg/ml and higher +S9. Relative survival after treatment –S9 was 96.2, 9.1, and 0.0% at 16, 32 and 64µg/ml, respectively, and +S9 was 74.2, 23.9, 17.3 and 0.0% at 16, 32, 64 and 128µg/ml respectively. Some outlier cytotoxicity was observed at 4µg/ml –S9 (81.2% relative survival). In the mutagenicity test, cell count toxicity was observed beginning at 8µg/ml ±S9; cytotoxicity in colony counts occurred at 16µg/ml and higher ±S9. Absolute survival was significantly decreased only in +S9 cultures at concentrations of 26µg/ml and higher. Relative survival after treatment was 59.3 and 13.2% at 16 and 21µg/ml –S9, and 59.8 and 34.5% at 16 and 64µg/ml+S9. . Number of mutants/10⁶ clonable cells –S9 was 13.7, 21.8, 22.5, and 0.5 at 4, 8, 16 and 21µg/ml respectively compared to controls (vehicle 22.7, medium 24.4) and +S9 was 1.0, 12.7, 5.3 and 17.7 at 16, 26, 32, and 64µg/ml, respectively compared to controls (vehicle 8.0, medium 14.7). There was no statistically significant increase in mutant frequency for any dose level of biphenyl feedstock compared to controls ±S9 in this assay Positive controls (EMS-S9 192.4 mutants/10⁶ clonable cells; B(a)P +S9 32.7 mutants/10⁶ clonable cells) and negative controls demonstrated appropriate responses .</p> <p>Biphenyl feedstock did not induce a mutagenic response with or without metabolic activation in CHO/HGPRT cells at any dose level. Cytotoxic effects were observed in both activated and non-activated cultures, demonstrating interaction of the test material with the cell system. Biphenyl Feedstock does not cause gene point mutations under the conditions of this assay.</p> <p>1. Reliable without restrictions.</p> <p>Papciak, R.J. and Goode, J.W. 1984. CHO/HGPRT test: using Biphenyl Feedstock. Proj. #2076. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX</p> <p>Hsie, A.W. et al. 1981. Mut. Res. 86: 193-214</p> <p>O'Neill, J.P. and Hsie, A.W. 1979. Banbury Report 2: 55-63</p> <p>Irr, J.D. and Snee, R.D. 1979. Banbury Report 2: 263-275.</p> <p>Snee, R.D., Smith, R.L., and Irr, J.D. 1981. MUTANT. A computer program for the evaluation of short-term mutation test results. E.I. Dupont de Nemours Co.</p> <p>Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel)</p> |
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Robust Summary - Group 10: Fuel Oils

Genetic Toxicity - in Vitro

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| <u>Test Substance</u> <i>Test substance</i> | Biphenyl Feedstock, CAS #68989-41-3. Amber liquid with aromatic odor. Composition analysis, purity and stability referred to sponsor. |
| <u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested | Standard method based on Williams et al. (1977,1982) In vitro mammalian cell DNA repair assay Unscheduled DNA Synthesis (UDS) in primary hepatocyte cultures. Yes 1984 Fischer 344 male rat (11-12 wks old) – 1 rat per test No NA NA NA Range-finding: 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048µg/ml: UDS assay 5.0, 20.0, 50.0, 100µg/ml; all diluted in 10% Pluronic [®] polyol F68 (prepared in deionized water, mol. wt 8350, 80% hydrophilic) |
| Exposure period Statistical Methods | 18.25-18.5 hours None employed. Criteria for positive response are incorporation of radioactive precursor (³ H-thymidine) in cells that are not normally synthesizing DNA, indicating repair of damage. A positive response is defined as a mean net nuclear grain count at any treatment level that exceeds concurrent negative control by at least 6 grains/nucleus; negative control value must not exceed 5 grains. A positive response need not be dose related. |
| Remarks for Test Conditions | Sufficient Biphenyl feedstock was weighed separately for each dose level and melted in an 85 ⁰ C water bath; 0.78ml of 10% F68 added per ml of final volume with sufficient balanced salt solution added to achieve final volume. Test preparations were stored at 50-60 ⁰ C until dosing when preparations were mixed and 30µl added to each 3ml culture. The conc. of ³ H-thymidine (½ life 12.5 yrs.) used in these assays was 1mCi/ml. All cultures were incubated at 37 ⁰ C in 5% CO2 enriched humidified atmosphere. For range-finding, primary hepatocytes derived from freshly perfused rat liver were seeded (approx. 1x10 ⁵ cells/ml) into treatment vessels, exposed to test material for 18.5 hours (2 cultures/dose level; 2 untreated cultures, and two vehicle F68 control cultures), then fixed in formalin and stained with trypan blue for viability determination. At least 50% viability needed for the assay. In the UDS assay, 1x10 ⁵ cells/ml were seeded into coverslip cultures, exposed to ³ H-thymidine and test substance for 18.25 hours (3 cultures/dose level). Positive control was 2-acetyl aminofluorene (2-AAF, 0.2µg/ml). Cells growing on coverslips were rinsed, fixed and glued to microscope slides on day 2. On day 3, slides were dipped in autoradiographic emulsion and stored in the dark at 2-8 ⁰ C. Autoradiographs were developed, stained and coverslipped on day 17. Number of grains overlying 50 randomly selected nuclei/slide were counted. The highest of 3 cytoplasmic grain counts/cell were subtracted to obtain net nuclear grain count. Avg. net nuclear grain count/slide (sum of net nuclear grain count ÷ 50) and mean net nuclear grain count (avg. net nuclear grain count/slide ÷ 3) were calculated. Test slides with negative average net nuclear grain counts were scored as zero. |
| <u>Results</u> Genotoxic effects | Biphenyl feedstock induced toxicity in primary hepatocytes following 18.5hrs exposure beginning at 8µg/ml (84.3% relative viability) with continuing decreases in viability with increasing dose to the maximum dose of 2048µg/ml (15% relative viability). Toxicity in the UDS assay occurred in the 50 and 100µg/ml dose groups resulting in fewer than 150 viable cells available for counting in each of these groups. Despite this toxicity, a positive, dose-related response for UDS was obtained at all dose levels. Mean net nuclear grain counts were 0.77, 0.00, 15.12, 68.46, 78.42, 251.91, and 248.28 for untreated medium control, vehicle F68 control, 5, 20, 50, 100µg/ml biphenyl feedstock, and 2-AAF positive control, respectively. |

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| <p><u>Conclusions</u> (contractor)</p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p> <p><u>Other</u> <i>Last changed</i></p> | <p>Biphenyl feedstock induced dose related unscheduled DNA synthesis in cultured rat hepatocytes at all doses evaluated. Biphenyl feedstock causes DNA damage and excision repair in this assay.</p> <p>1. Reliable without restrictions. Study conforms to standard design. GLPs have been followed.</p> <p>Brecher, S., Goode, J.W. 1984. Hepatocyte primary culture/DNA repair test of Biphenyl feedstock. Proj. #2078. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX</p> <p>Williams, G.M. 1977. Cancer Res. 37: 1845-1851</p> <p>Williams et al. 1977. In Vitro 13: 809-817</p> <p>Williams et al. 1982. Mut. Res. 97:359-370</p> <p>6/10/2001 (Prepared by a contractor to the Olefins Panel)</p> |
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Robust Summary - Group 10: Fuel Oils

Genetic Toxicity - in Vitro

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| <u>Test Substance</u> <i>Test substance</i> | Biphenyl Feedstock, CAS #68989-41-3. Amber-colored liquid with aromatic odor. Compositional analysis, purity and stability referred to sponsor. |
| <u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested | Standard method based on Cortesi et al (1983), Dunkel et al (1981), Reznikoff et al (1973) In vitro cell transformation Mouse embryo cells Yes 1983 BALB/3T3-A31-1-1 from T. Kakunaga, National Cancer Inst., 1982 No NA NA NA Cytotoxicity: 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048µg/ml; Transformation: 4, 8, 16, 32µg/ml, all diluted in 10% Pluronic [®] polyol F68 (prepared in deionized water, mol. wt. 8350, 80% hydrophilic). |
| Exposure period Statistical Methods | 2 days None employed. Criteria for positive response were a two-fold increase in type III foci at the highest dose over vehicle control (at least 2 type III foci if vehicle control had none) with or without a dose related response, or a two-fold increase at two or more consecutive doses. Test is equivocal if two-fold increase occurred at any one level other than the highest acceptable dose. |
| Remarks for Test Conditions | Sufficient Biphenyl feedstock was weighed separately for each dose level and melted in an 85 ⁰ C water bath; 0.78ml of 10% F68 added per ml of final volume and balanced salt solution was added as required to achieve final volume for testing. Test preparations were stored at 50-60 ⁰ C until just prior to dosing when the preparations were mixed and added at 50µl to each 5 ml culture. All cultures were incubated at 37 ⁰ C in 5% CO ₂ enriched humidified atmosphere. For cytotoxicity, 2 flask cultures/dose group, 2 cultures for vehicle F68 or medium negative control were seeded with 1x10 ⁴ cells/culture in day 1, exposed on days 2-3, trypsinized and counted with a Coulter Model ZB on day 4 for at least 20% survival. For transformation, 15 flask cultures (1x10 ⁴ cells/culture/dose group)) and two colony formation flask cultures (100 cells/culture/dose group) were seeded on day 1, exposed on days 2-3 and culture medium changed on day 4. For transformation cultures, medium continued to be changed weekly to day 29. Positive control was 3-methylcholanthrene (1µg/ml). Colony formation cultures were fixed, stained, and counted visually on day 8 to determine cloning efficiency (avg. number colonies/flask ÷ 100 cells seeded). Transformation cultures were fixed and stained on day 29 for focus counting and evaluation. Transformation frequency = total type III foci ÷ total flasks/dose group. |
| <u>Results</u> Genotoxic effects | Biphenyl feedstock induced toxicity in BALB/3T3 cells after two days exposure beginning at 16µg/ml (49% viability); inducing reduction to 20% viability between 32-64µg/ml and 2.1% viability at 2048µg/ml. In the transformation assay, a progressive increase in cytotoxicity occurred with increasing doses from 8-32µg/ml reducing the relative cloning efficiency (rel. C.E.) from 72.5-27.5%, respectively. At 32µg/ml, the toxic response of 27.5% rel. C.L. was comparable to that of the positive control, 3-methylcholanthrene (28.4% rel. C.E.). The positive control induced the expected response for transformation: 10 type III foci. The vehicle control had 1 type III focus, but the untreated medium control was slightly higher with 2 type III foci. The 8µg/ml and 32µg/ml biphenyl feedstock cultures each had 2 type III foci compared with one type III focus in the vehicle control. However, since the untreated control also had 2 type III foci, the results from treated cultures were considered negative. |
| <u>Conclusions</u> | Biphenyl feedstock did not induce significant transformation in BALB/3T3 cells under conditions of this assay |

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| <p>(contractor)</p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p> <p><u>Other</u> <i>Last changed</i></p> | <p>conditions of this assay.</p> <p>1. Reliable without restriction.</p> <p>Brecher, S, and Goode, J.W. 1983. BALB/3t3 transformation test: Biphenyl Feedstock. Proj. #2079. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co, Houston, TX</p> <p>Cortesi, E. et al. 1983. Teratogenesis, Carcinogenesis, Mutagenesis 3: 101-110.</p> <p>Dunkel, V.A. et al. 1981. J. Nat'l Cancer Inst. 67: 1303-1315.</p> <p>Reznikoff, C.A. et al. 1973. Cancer Res. 3239-3249.</p> <p>6/07/2001 (prepared by a contractor to the Olefins Panel).</p> |
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Robust Summary - Group 10: Fuel Oils

Genetic Toxicity - in Vivo

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| <u>Test Substance</u> | Biphenyl Feedstock, CAS #68989-41-3. Amber-colored liquid with aromatic odor. |
| Remarks | Compositional analysis, purity and stability referred to sponsor. |
| <u>Method</u> | |
| Method/guideline followed | Comparable to standard assay |
| Type | Mammalian bone marrow erythrocyte micronucleus |
| GLP | Yes |
| Year | 1984 |
| Species | Mouse |
| Strain | CrI:CD [®] -1 (ICR) BR Swiss |
| Sex | Male and female: 10M, 10F/group; 15M, 15 F in 1 group |
| Route of administration | Oral gavage |
| Doses/concentration levels | 0, 0.25, 0.5, 1.0g/kg in corn oil |
| Exposure period | 1 dose/day for 2 days; 1 group- 1 dose, 1 day only |
| Statistical methods | Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes (PCEs) with micronuclei (MN) , and group mean ratios of PCE to normochromatic erythrocytes (NORMs) were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant (p<0.05) increases in micronucleated PCE at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met. |
| Remarks for Test Conditions. | Biphenyl feedstock dosing solutions were prepared fresh for each day of dosing—2.5 g was mixed with corn oil to make 50 ml, blended by shaking. In an initial study at doses of 0.0, 0.75, 1.5, 2.0, and 3.0g/kg, very high mortality occurred at doses of 1.5-3.0g/kg and the study was terminated. A new study was initiated at doses of 0.25–1.0g/kg in a single dose daily by gavage for 2 days. All mice were weighed on day 1 and on day of sacrifice. One half of each treated group and vehicle control (5M, 5F) was killed on day 3 and the remainder on day 4. One group (15M, 15F), given 1.0 g/kg by gavage in a single dose for 1 day only, was killed on days 2, 3, 4 (5/sex/day). Positive control mice given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Slides of femoral bone marrow smears were prepared, stained with May-Grunewald /Giemsa stain and examined microscopically. For each mouse, 1000 PCE and all associated mature erythrocytes (NORMs) were counted. Data collected included group mean body weights for each day, total PCEs, total NORMs, PCEs with MN, and NORMs with MN. |
| <u>Results</u> | |
| Genotoxic effects | No mortality occurred at any dose level and no effects on body weight were observed in either sex. Mice treated with biphenyl feedstock did not show any significant change in the frequency of micronucleus formation in PCE and no significant changes in the ratio of PCE/NORM compared to vehicle controls. Positive and negative controls performed appropriately. |
| NOAEL (NOEL) | NOEL(genetic and systemic) = 1.0g/kg |
| LOAEL (LOEL) | |
| <u>Conclusions</u> | |
| (study authors) | Oral treatment of mice with Biphenyl feedstock for 1-2 days at doses up to 1.0 g/kg/day had no effect on frequency of micronucleated polychromatic erythrocytes in bone marrow. Under these test conditions LPFO does not induce cytogenetic damage. |
| <u>Data Quality</u> | |
| Reliabilities | 1. Reliable without restrictions. Study conforms to standard design. GLP have been followed. |
| <u>References</u> | |
| | Khan, S.H. 1984. Micronucleus test in mouse bone marrow: Biphenyl feedstock administered orally for 2 days. Proj. #2077. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX |
| <u>Other</u> | |
| Last changed | 6/07/2001 (Prepared by a consultant to the Olefins Panel) |

Robust Summary - Group 10: Fuel Oils

Repeated Dose Toxicity

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| <u>Test Substance</u> | Biphenyl Feedstock, CAS #68989-41-3. Dark brown liquid with crystal aggregates. Refer to sponsor for further information |
| Remarks | |
| <u>Method</u> | |
| Method/guideline followed | None specified. Comparable to standard study |
| Test type | Sub-acute |
| GLP | Yes |
| Year | 1983 |
| Species | Rat, |
| Strain | Fischer 344 |
| Route of administration | Dermal |
| Duration of test | 12 days |
| Doses/concentration levels | 0, 1.0, 2.0g/kg (diluted in corn oil to constant volume of 4ml/kg for administration) |
| Sex | 5M, 5F/dose group |
| Exposure period | 6hr/day |
| Frequency of treatment | days 1-5, 8-11 |
| Control group and treatment | 5M, 5F; 4ml corn oil/kg |
| Post exposure observation period | None |
| Statistical methods | Mean and standard deviation, Bartlett's test, and analysis of variance. Dunnett's test if data were homogeneous; modified t-test if data were non-homogeneous. |
| Test Conditions | Rats were housed individually in suspended, stainless steel cages with mesh fronts and bottoms, equipped with an automatic watering system. Water and rat chow diet were available ad lib. Room temperature was maintained at 74°F with a relative humidity of 55% and 12 hour light/dark cycle. Backs of rats were clipped of hair and dosed dermally on days 1-5 and days 8-11 at 0, 1.0, and 2.0g/kg diluted in corn oil. Elizabethan collars were applied for the 6 hour exposure period, after which collars were removed and skin wiped free of test article. Rats were sacrificed on day 12. Rats were observed for mortality and moribundity twice daily on dosing days and once daily on non-dosing days. Body wt was recorded 5 days before initiation and on days 1, 8, and before sacrifice on day 12. Rats were observed once daily for clinical signs on dosing days. Dermal effects were scored twice on each dosing day - before application and after removal. Blood was taken for clinical chemistry and hematology 7 days prior to initiation and on day 12 prior to sacrifice; parameters were total leukocyte count, total erythrocyte count, total platelets, hemoglobin, hematocrit, mean cell vol., mean cell hemoglobin, mean corpuscular hemoglobin, BUN, creatinine, alkaline phosphatase, sodium, potassium, glucose, SGOT, SGPT, total protein, albumin, and albumin/globulin ratio. All animals received a gross necropsy and potential target organs/tissues (liver, brain, heart, spleen, kidneys, testes) were weighed and preserved for histopathology. Certain organs/tissues (lung, skin, ovaries) were examined microscopically but not weighed. Only control and high dose groups were scheduled for histopathology. |
| <u>Results</u> | |
| NOAEL (NOEL) | LOEL = 1g/kg based on reduction in body wt. (Assigned by reviewer) |
| LOAEL (LOEL) | No mortality or moribundity was observed during the study. Body wts of males and females in groups 2 and 3 were reduced but the effect was statistically significant only in females. |
| Remarks | No dermal reactions or test article related clinical signs were observed. There were no biologically significant test article related changes in the hematology or clinical chemistry parameters. However, sera of test article-treated rats were more yellow than that of controls. There was an increase in absolute wt. of kidneys of males and females in the test article groups that was significant only for female left kidney. There were no gross pathological changes in skin at the site of application. There were no histopathological lesions that could be attributable to test article exposure. |
| <u>Conclusions</u> (study authors) | Dosing with the test article did not produce overt toxicological effects but there were decreases in terminal body wt and increases in specific organ wt that appeared to be treatment related |

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| <p><u>Quality</u> Reliabilities</p> <p><u>References</u></p> <p><u>Other</u> Last changed</p> | <p>2. Reliable with restrictions. Dosing solution concentrations were not verified by analysis.</p> <p>Rausina, G.A. 1983. Two-week repeated dose toxicity study in rats using Biphenyl Feedstock. Proj. #2044. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX</p> <p>6/07/2001 (Prepared by a contractor to the Olefins Panel)</p> |
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Robust Summary - Group 10: Fuel Oils

Repeated Dose Toxicity

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| <u>Test Substance</u> | Biphenyl Feedstock, CAS #68989-41-3. Amber-colored, semi-solid with aromatic odor. |
| Remarks | Refer to sponsor for further information. |
| <u>Method</u> | |
| Method/guideline followed | None specified. Comparable to standard study |
| Test type | Sub-acute |
| GLP | Yes |
| Year | 1983 |
| Species | Rat |
| Strain | Fischer 344 |
| Route of administration | Inhalation |
| Duration of test | 12 days |
| Doses/concentration levels | 0, 1.07, 3.04g/m ³ |
| Sex | 5M, 5F/dose group |
| Exposure period | 6 hr/day |
| Frequency of treatment | days 1-5, 8-11 |
| Control group and treatment | 5M, 5F; filtered air |
| Post exposure observation period | None |
| Statistical methods | Analysis of variance, Dunnett's test, Kolmogorov-Smirnov two-tailed test |
| Test Conditions | <p>Rats were housed individually in stainless steel screen-bottomed cages in a room maintained at 75°F with 37% relative humidity and 12 hour light/dark cycle. Chow diet and water were provided ad lib except during exposure. Test substance was aerosolized with a ball jet nebulizer heated to 70°C to decrease viscosity. Chamber concentrations were determined by fluorescence spectroscopy. Both vapor phase and particulate phase concentrations were determined. Actual total exposure (and % of total as particulate) were 0, 1.07g/m³ (60%) and 3.04g/m³ (51%). Particulate mass median aerodynamic diameter was 5.2-5.4 microns. Rats were exposed to test article or filtered air for 6hr/day on days 1-5 and days 8-11; they were observed twice daily on dosing and once on non-dosing days for mortality and moribundity, and immediately after dosing for clinical signs. Body wt was recorded immediately prior to exposure on days 1 and 5, and prior to sacrifice on day 12. Non-fasted blood was collected on day 12 for blood chemistry and hematology. Measured chemistry parameters were BUN, creatinine, total protein, albumin, albumin/globulin ratio, and SGPT. Hematology parameters were white blood cells, red blood cells (RBC), hemoglobin, hematocrit, mean RBC cell vol., mean RBC corpuscular vol., and mean corpuscular hemoglobin. All rats were necropsied for gross lesions. Organs weighed were liver, brain, heart, kidneys, spleen, testes, and lungs; these organs as well as ovary, uterus, eyes and nasal turbinates were saved for histopathological examination. Only organs/tissues from control and high dose rats were examined.</p> |
| <u>Results</u> | |
| NOAEL (NOEL) | LOEL = 1.07g/m ³ based on body wt loss. Assigned by reviewer. |
| LOAEL (LOEL) | There were no test article-related deaths during the study. Males and females of groups 2 and 3 showed dose related weight loss. Most of the group 2 and 3 rats showed perianal soiling, excessive ocular porphyrin, dry red matter around mouth and nose, and crystalline test article on fur. There were no biologically significant effects of test article on clinical chemistry or hematology parameters. However, there was a dose related yellowing of blood sera. There were significant increases in absolute liver and kidney wt of high dose females, and decreases in spleen wt of high dose males. There were many significant increases in organ/body wt ratios owing to decreased wt gain in treated rats. There were no microscopic changes in male or female organs/tissues attributable to test article exposure. The only effect noted at gross necropsy was skin discoloration in test article treated rats. |
| Remarks | |
| <u>Conclusions</u> | |
| (study authors) | Repeated exposure to 1.07g/m ³ or 3.04g/m ³ of test article caused no mortality. Several exposure-related clinical effects were observed including perianal soiling, respiratory and ocular discharges, and decreased body wt. In addition, at the high dose, female liver and |

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| <u>Quality</u> Reliabilities <u>References</u> <u>Other</u> Last changed | kidney wts were increased and male spleen wt was decreased. 1. Reliable without restrictions Gordon, T. 1983. Nine-day repeated dose inhalation study in rats, Biphenyl Feedstock. Proj. #2045. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX 6/07/2001 (Prepared by a contractor to the Olefins Panel) |
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Robust Summary – Group 10: Fuel Oils

Invertebrate Acute Toxicity

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| <u>Test Substance</u> | Biphenyl feedstock, CAS #68989-41-3 |
| <u>Method</u> | |
| Method/guideline followed | None specified. Comparable to standard methods |
| Year (guideline) | Unknown |
| Type (test type) | Acute Daphnid Flow-through Toxicity Study |
| GLP | Yes |
| Year (study performed) | 1984 |
| Species | Water flea (<i>Daphnia magna</i>) |
| Analytical Monitoring | Yes (total organic carbon) |
| Exposure Period | 48 hours |
| Statistical Methods | Probit procedure of SAS. Chi square on each dose-response curve to verify non-heterogeneity and goodness of fit. |
| Test Conditions | First instar (24-hour old) offspring of 20 gravid <i>Daphnia</i> were used as test animals in a flow-through proportional diluter system. One day prior to introduction into the flow-through system, 1000 mg/L nominal concentration of biphenyl feedstock was prepared by mixing 14.4 g of test material into 14.4 L water. The stock solution was continuously stirred while being dispensed into the diluter. Test concentrations of 0, 10, 18, 32, 56, 100 mg/L (nominal) were produced by dilution with volumes of control water. Flow rate through the system was 14.4 L of test preparation dispensed every 24 hours (25 ml dispensed during each 2.5 min/cycle. The flow-through system delivered 100 ml test solution to each dose level every 2.5 min/cycle (50 ml into each 3.0 L capacity duplicate flask). Starting at one end of the bioassay table, 2 daphnids were placed into a floating polypropylene cylindrical container with a mesh bottom in each test vessel (2 vessels/dose) in consecutive order to the end of the table, procedure was repeated going in reverse direction, then back again until each vessel contained 10 daphnids. Use of the cylindrical container in the test vessel allowed water to pass through, improved observation of the animals and prevented daphnids from escaping and being accidentally discharged at the overflow standpipe. All acclimation and test vessels contained charcoal filtered municipal water, pH 7.8-8.5, at a temperature range of 18.4-20.3°C and daily photoperiod of a continuous 12 hour light/dark cycle. Daily records were kept on number of immobilized and dead animals/vessel at 6, 24, and 48 hours. Water temperature was recorded daily. Dissolved oxygen conc. (9.3-10.1 mg/L), pH, total alkalinity (31 mg/L as CaCO ₃) specific conductivity (297-311 umhas/cm), and hardness (80-82 mg/L as CaCO ₃) were measured during the first 6 hours, and at 48 hours in each vessel. Water samples for chemical analysis of actual concentration by fluorescence spectrometer (410 nm wavelength) were collected on day 0 and at 48 hours from each bioassay vessel. Actual concentration was calculated by subtracting the mean background TOC value of 6.8 mg/L from analytical test material concentrations at each dose level. The positive control compound, hexavalent chromium as potassium dichromate was detected in water samples by atomic absorption spectrometry. |
| <u>Results</u> | |
| Units/Value: | EL ₅₀ (24-hour) = > 100 mg/L (95% Confidence limits not determined) based on nominal concentrations |
| Note: Deviations from protocol or guideline, analytical method, biological observations, control survival | EL ₅₀ (48-hour) = 23.6 mg/L (95% Confidence limits = 17.5 - 30.7 mg/L) based on nominal concentrations Percent mortality at 48 hours was 0, 22, 39, 44, 89, 100% for untreated control (0), and nominal concentrations of 10, 18, 32, 56, 100 mg/L, respectively. Actual water soluble concentrations of biphenyl feedstock were 0.1, 1.7, 2.7, 3.8, and 7.5 mg/L at nominal concentrations of 10, 18, 32, 56, 100 mg/L, respectively. No treatment related changes in any water characteristic measurement were induced by test material or positive control. The 24-hour and 48-hour EC ₅₀ values for hexavalent chromium were 0.18 and 0.13 mg/L |

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| <p><u>Conclusions</u> (study author)</p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p> <p><u>Other</u> <i>Last changed</i></p> | <p>The 24-hour and 48-hour EC₅₀ values for hexavalent chromium were 0.18 and 0.13 mg/L, respectively.</p> <p>(1) Reliable without restriction</p> <p>Meyers, W.R., Rausina, G.A. 1984. 48-hour Aquatic Toxicity Study in <i>Daphnia</i> with Biphenyl Feedstock. Project #2042. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemical Co., Houston, TX, USA.</p> <p>American Chemistry Council, Olefins Panel</p> |
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Robust Summary – Group 10: Fuel Oils

Biodegradation

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| <p><u>Test Substance</u></p> <p><u>Method</u></p> <p>Method/guideline followed Year (guideline) Type (test type) GLP Year (study performed) Inoculum Exposure Period Test Conditions</p> <p>Note: Concentration prep., vessel type, replication, test conditions.</p> <p><u>Results</u></p> <p>Units/Value:</p> <p>Note: Deviations from protocol or guideline, analytical method.</p> <p><u>Conclusions</u> (study author)</p> <p><u>Data Quality</u> Reliabilities</p> <p><u>References</u></p> | <p>Biphenyl Feedstock, CAS #68989-41-3</p> <p>OECD guideline 301D; EEC directive 67/548 Annex V part C.6 (84/449/EEC) 1984 Aerobic Aquatic Biodegradation (Closed Bottle Test) Yes 1993 Domestic activated sewage sludge bacteria from Huntingdon Research Centre sewage treatment plant 28 days</p> <p>Biphenyl feedstock was initially dissolved in chloroform to give a stock solution of 560 mg/ml; 10 ml aliquots were adsorbed on Whatman GFA glass filter paper and solvent evaporated to dryness. One piece of filter paper was placed in each test bottle prior to filling with inoculated medium. Test bottles included non-inoculated nutrient medium, inoculated nutrient medium, inoculated medium plus blank filter paper, 2 mg/L biphenyl feedstock adsorbed on filter paper, 3 mg/L sodium benzoate standard, and 2mg/L biphenyl feedstock adsorbed on filter paper +3 mg/L sodium benzoate to evaluate inhibitory effects. The nutrient medium consisted of aerated reverse osmosis purified, deionized water, phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride. Activated sewage sludge filtrate was added at a rate of 1 drop of inoculum/liter. Biochemical oxygen demand (BOD) dark glass 280 ml culture bottles, fitted with ground glass stoppers, were filled by siphon and firmly stoppered to exclude all air bubbles. Duplicate bottles were prepared in each test and control series to allow single oxygen determination/bottle at 0, 5, 15, and 28 days. The bottles containing biphenyl feedstock on filter paper +sodium benzoate were sampled on day 0 and 28. All bottles were incubated in a water bath at $20\pm 1^{\circ}\text{C}$; measurements of dissolved oxygen conc. were made with a Yellow Springs BOD meter. Concentrations of biphenyl feedstock or sodium benzoate as mg carbon/L were not provided. Chemical oxygen demand (COD) was determined using a semi-micro sample digestion method. Test substance samples (50 μl) dissolved in chloroform were placed in clean, dry reaction vials and evaporated to dryness. Deionized water (2 ml), + sulfuric acid, potassium dichromate, mercuric sulfate, and silver catalyst were added and reaction vial heated at 150°C for 2 hours. COD values were read by spectrophotometer. Control blanks using solvent only and a sodium benzoate standard were also evaluated.</p> <p>Percentage biodegradation was determined by comparing the oxygen depletion value with the corresponding Theoretical oxygen demand (NO_3)[$\text{ThOD}_{(\text{NO}_3)}$]. $\text{ThOD}_{(\text{NO}_3)}$ was 2.94 mgO_2/mg for biphenyl feedstock (supplied by sponsor) and 1.67 mgO_2/mg for sodium benzoate. Chemical oxygen demand (calculated) for biphenyl feedstock was slightly lower at 2.38 mgO_2/mg; for sodium benzoate COD was 100% $\text{ThOD}_{(\text{NO}_3)}$. Biphenyl feedstock attained 57% biodegradation within 28 days. Sodium benzoate degraded 86% within 28 days. Cultures containing both biphenyl feedstock and sodium benzoate showed an oxygen depletion value 4% higher than anticipated on the basis of separate cultures indicating that test material does not have an inhibitory effect on sewage bacteria. No inhibitory effects on activated sewage bacteria were observed in this assay.</p> <p>(1) Reliable without restriction</p> |
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Robust Summary – Group 10: Coal-Derived Fuel Oils

Acute Toxicity

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| <u>Test Substance</u> | EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538 ⁰ C. |
| <u>Method</u> | |
| Method/guideline followed | None specified |
| Type (test type) | Acute limit test |
| GLP | Yes |
| Year | 1984 |
| Species/Strain | Rat/ Sprague-Dawley |
| Sex | Male and female |
| No.animals/sex/dose | 5 |
| Vehicle | None |
| Route of administration | Oral |
| Test Conditions | Animals were individually caged and maintained on a 12 hour light/dark cycle, at approximately 70 ⁰ F and 40% relative humidity. Food and water were provided ad lib. Rats were 6-12 weeks of age at study initiation. A single administration of 5 g/kg was given by gavage. Animals were observed for 14 days post-dose. Gross necropsy was conducted at termination. |
| <u>Results</u> | |
| LD ₅₀ with confidence limits. | LD ₅₀ > 5 g/kg. |
| Remarks | Three of the dosed rats died during the post-dosing period (Days 2, 3, and 6). Clinical signs observed in the surviving rats included ataxia, alopecia, abdominal griping, nasal discharge, urinary, fecal, and ano-genital staining, unthrifty coat, hypoactivity, wet and dry rales, and ocular discharge. The surviving rats all gained weight throughout the observation period. There were no significant findings at gross necropsy. |
| <u>Conclusions</u> (study author) | The test material was judged to be essentially non-toxic. |
| <u>Data Quality</u> | |
| Reliability | 1. Reliable without restrictions. |
| <u>References</u> | McKee, R.H., Biles, R.W., Kapp, R.W. and Hinz, J.P. 1984. The acute toxicity of coal liquefaction-derived materials. J. Appl. Toxicol. 4: 198-205. |
| <u>Other</u> Last changed | 14-Dec-01 (Prepared by a contractor to the Olefins Panel). |

Robust Summary - Group 10: Coal-Derived Fuel Oils

Acute Toxicity

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| <u>Test Substance</u> | EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538 ⁰ C. |
| <u>Method</u> | NAS procedure, Draize scoring |
| Method/guideline followed | Acute dermal toxicity |
| Type (test type) | Yes |
| GLP | 1984 |
| Year | Rabbit/New Zealand White |
| Species/Strain | Males and females |
| Sex | 6 |
| No. of animals/sex/dose | None |
| Vehicle | Dermal |
| Route of administration | |
| Test Conditions | The study was designed to assess potential for systemic toxicity and skin irritation. Animals were individually caged and maintained on a 12 hour light/dark cycle, at approximately 70 ⁰ F and 40% relative humidity. Food and water were provided ad lib. Rabbits were about 12 weeks of age at study initiation. A single administration of 3.16 g/kg was applied to the clipped dorsal surface of the back of 6 rabbits (3 with abraded backs, 3 non-abraded), and covered with a gauze patch under a plastic sleeve. Patches were removed after 24 hours. Observations were made at 24 hrs post-dose and then daily for a total of 14 days; dermal responses were evaluated 30 min. after dosing, and at 2, 3, 7, 10 and 14 days post-dose. A gross necropsy was performed at terminal sacrifice. |
| <u>Results</u> | |
| LD ₅₀ with confidence limits. | LD ₅₀ > 3.16 g/kg. |
| Remarks | No animals died during the study. Clinical signs observed during the 14-day observation period included emaciation, nasal discharge, soft stool, and alopecia. Dermal observations included severe erythema and moderate edema; positive dermal scores persisted to study termination. Rabbits also exhibited atonia, fissuring, exfoliation, eschar and desquamation. Both males and females exhibited slight weight loss from study initiation to Day 14. |
| <u>Conclusions</u> (study author) | The test material produced significant dermal irritation and skin injury as evidenced by fissuring, desquamation, and eschar formation. |
| <u>Data Quality</u> Reliability | 1. Reliable without restrictions |
| <u>References</u> | McKee, R.H., Biles, R.W., Kapp, R.W. and Hinz, J.P. 1984.. The acute toxicity of coal liquefaction-derived materials. J. Appl. Toxicol. 4: 198-205. National Academy of Sciences, 1977. Principles and procedures for evaluating the toxicity of household substances. Pp. 23-57. Washington, DC Draize, J.H. et al. 1944. Pharmacol. Exp. Therapeut. 82: 377 –390. |
| <u>Other</u> Last changed | 14-Dec-01 (Prepared by a contractor to the Olefins Panel). |

Robust Summary - Group 10: Coal-Derived Fuel Oils

Genetic Toxicity - in Vitro

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| <u>Test Substance</u> <i>Test substance</i> | EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538 ⁰ C. |
| <u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested Statistical Methods | Standard method per Ames et al. 1975, 1983 Reverse mutation bacterial assay Salmonella typhimurium with and without metabolic activation Yes. 1984 (1995 publication) Salmonella typhimurium TA100, TA98 Yes Sprague-Dawley rat or Syrian Golden hamster liver (S9 fraction), sex not specified 50ul S9 fraction/plate (0.1ml S9 fraction/1.0ml S9 mix) Aroclor 1254 induced; dosage and treatment not specified 0.0, 0.1, 1.0, 10.0, 50, 100, 500µg/ml ±S9, diluted in dimethyl sulfoxide (DMSO); additional doses with Tween 80 dispersant were 1000, 10,000µg/ml. Mutation induction curve based on revertants/plate was plotted and mutagenic potency, the slope of revertants/µg dose of each induction curve was determined by non-linear regression analysis |
| Remarks for Test Conditions | Assays were conducted only in strains TA 100 and TA98 ±S9, the strains potentially most sensitive to detect activity by this type of complex hydrocarbon. Positive results were observed only in TA98+S9 and are the only data reported. Fresh bacterial stocks were exposed to graded doses of test material ±S9 from rat or hamster liver S9 (3 plate/dose/ liver fraction). Positive control was benzo(a)pyrene (5µg/plate); vehicle controls were DMSO or Tween 80. When detergent dispersant was utilized, equal volumes of test material and Tween 80 were mixed, distilled water was added dropwise to produce emulsions with final concentration of 10%(v/v) oil. Additional dilutions required for testing were made with 10% solutions of Tween 80 in distilled water to maintain constant detergent concentrations in test samples. Aliquots of these dilutions were plated with TA100 and TA98 ±S9 from rat or hamster liver. After 72 hr incubation at 37 ⁰ C, revertant colonies were counted with a Biotran III automatic colony counter. |
| <u>Results</u> Genotoxic effects | Fuel Oil blend produced a dose related increase in revertant frequency under all testing conditions in strain TA98+S9. Mutagenic potency was enhanced when hamster S9 was employed vs rat S9 (7.22 vs 5.29 revertants/µg, respectively). Detergent dispersion did not increase assay sensitivity for the fuel oil blend. Positive and negative controls performed appropriately; hamster S9 activation improved response of bacteria to benzo(a)pyrene but addition of dispersant did not enhance response beyond hamster S9 alone. |
| <u>Conclusions</u> (study authors) | EDS fuel oil blend was the most active bacterial mutagen of the high boiling coal liquids tested in this assay and contained the highest level of nitrogen. These positive results agree qualitatively with positive results in dermal carcinogenesis studies; however the lack of quantitative agreement may be related to the fact that the Salmonella assay is highly sensitive to aromatic amines and nitroaromatic compounds while dermal carcinogenesis is predominantly associated with neutral polycyclic aromatic hydrocarbons. Coal derived liquids containing substantial amounts of materials boiling above 370 ⁰ C are active in carcinogenesis screening assays. |
| <u>Data Quality</u> Reliability | 1. Reliable without restrictions. |
| <u>Reference</u> | McKee, R.H., Traul, K.A., and Przygoda, R.T. 1995. Evaluation of coal liquids derived from EDS process in carcinogenesis screening tests. J. Appl. Toxicol. 15: 159-165 (see separate summary for cell transformation data) Ames, B.N. et al. 1975. Mutat. Res. 31: 347-364 Maron, D.M. and Ames, B.N. 1983. Mutat Res. 113: 173-215. |
| <u>Other</u> Last changed | 14-Dec-01 (Prepared by a contractor to the Olefins Panel). |

Robust Summary - Group 10: Coal-Derived Fuel Oils

Genetic Toxicity - in Vitro

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| <u>Test Substance</u> | EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538 ⁰ C. |
| <u>Method</u> | |
| Method/guideline followed | Standard method per Pienta et al, 1977; Przygoda et al, 1985 |
| Type | Cell transformation |
| System of testing | Syrian Hamster embryo cells (SHE) |
| GLP | Not specified |
| Year | 1984 (1995 publication). |
| Species/Strain | Cells isolated from eviscerated, decapitated day 13 embryos of timed-pregnant Syrian hamsters from Eagle Laboratories, Farmersburg, IN. |
| Metabolic activation | No |
| Species and cell type | NA |
| Quantity | NA |
| Induced or not induced | NA |
| Concentrations tested | 0.0, 0.1, 0.5, 1.0, 5.0, 10.0, 50, 100, 500, 1000µg/ml, diluted in dimethyl sulfoxide (DMSO) |
| Statistical Methods | None |
| Remarks for Test Conditions | Primary cultures of freshly derived hamster embryo cells were grown on Dulbecco's modified Eagles medium with 20% fetal bovine serum for 2 days, then frozen. Each assay consisted of concurrent tests in two distinct pools of embryo cells (each pool comprised of all cells from a single litter). Cell pools were screened with mutagenic and non-mutagenic compounds for validation prior to study initiation. Irradiated feeder cells were plated in 60 mm culture dishes; 24 hr later, non-irradiated target cells were plated at density of 300 cells/dish. One day later, test material was added to 3 plates/concentration/cell pool. Cells were grown for 7 days, then fixed and stained, and transformed colonies identified and counted. Total colony count and number of transformed colonies were recorded for each plate. Cloning efficiency and transforming efficiency were calculated. Assay was valid only if spontaneous and vehicle control cultures had no spontaneous transformed foci and the positive control, benzo(a)pyrene induced transformation at 2 doses (5, 10µg/ml). |
| <u>Results</u> | |
| Genotoxic effects | Morphological transformation in SHE cells was induced over a concentration range of 1-100µg/ml and was toxic to concentrations exceeding 100µg/ml. Results were comparable in both cell pools. Positive and negative controls performed appropriately. |
| <u>Conclusions</u> (study authors) | EDS experimental fuel oil blend induced transformation in SHE cells with approximately equal efficiency compared to two other coal derived high boiling liquids tested concurrently. Both neutral PAH and nitrogen-containing PAH fractions are active in this assay. The results of this assay agree with positive dermal carcinogenicity studies and demonstrate that coal derived liquids containing substantial amounts of materials boiling above 370 ⁰ C are active in carcinogenesis screening assays. |
| <u>Data Quality</u> | |
| Reliability | 1. Reliable without restriction. |
| <u>References</u> | McKee, R.H., Traul, K.A., and Przygoda, R.T. 1995. Evaluation of coal liquids derived from EDS process in carcinogenesis screening tests. J. Appl. Toxicol. 15: 159-165. Pienta, J.A. et al. 1977. Int. J. Cancer 19: 642-655 Przygoda, R.T. et al. 1985. In Vitro 21: 32-38 |
| <u>Other</u> | |
| Last changed | 14-Dec-01 (Prepared by a contractor to the Olefins Panel). |

Robust Summary - Group 10: Coal-Derived Fuel Oils

Repeated Dose Toxicity

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| <u>Test Substance</u> | EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538 ⁰ C. |
| <u>Method</u> | Other. |
| Method/guideline followed | 28-day dermal. |
| Test type | Yes |
| GLP | 1983 (1985 publication) |
| Year | Rabbit |
| Species | New Zealand White |
| Strain | Dermal |
| Route of administration | 4 weeks |
| Duration of test | 0, 50, 200 mg/kg |
| Doses/concentration levels | Males and females (5/sex/dose) |
| Sex | 4 weeks |
| Exposure period | 5 days/wk |
| Frequency of treatment | 5M, 5F; Primol 185 (highly purified mineral oil; white oil) |
| Control group and treatment | None |
| Post-exposure observation period | Dunnett's test for comparing means of test groups and controls |
| Statistical methods | |
| Test Conditions | Rabbits (3-5kg) were individually housed in suspended steel cages in a room maintained between 18-22 ⁰ C, relative humidity of 40-70% and a 12 hr light/dark cycle. Food and water was available ad lib. Test material was applied to unabraded skin in an area of approx. 200cm ² on the dorsal surface between the shoulders and lumber region. Elizabethan collars were used to minimize ingestion. Test material (50 and 200mg/kg) was diluted in Primol 185, and administered at 2ml/kg for 5 consecutive days/wk during the 4 wk dosing period. Residual test article was allowed to remain or accumulate on the skin. The 200mg/kg dose selected was the highest dose that did not cause weight loss in a 5 day range-finding study. Rabbits were clipped twice weekly during the 4 wk dosing period. Rabbits were observed daily for clinical signs of toxicity and skin irritation. Body weight and food consumption were determined weekly. Blood was collected for hematology and clinical chemistry from unfasted rabbits prior to dosing, and at sacrifice (24 hr fasted). All animals were necropsied and organ wt determined for liver, kidney, epididymides and testes. Tissue preserved for histopathological examination included brain, heart, lungs, liver, kidney, spleen, testis, epididymis, prostate, seminal vesicle, ovary, urinary bladder, adrenal, pancreas, thymus, bone marrow, and skin. |
| <u>Results</u> | |
| NOAEL (NOEL) | NOAEL not determined |
| LOAEL (LOEL) | LOAEL = 50mg/kg based on body weight reduction, liver weight increase, serum cholesterol increase (estimated by reviewer). |
| Remarks | No mortality occurred during the study. At the site of application, desquamation, blanching, atonia and fissuring were observed in the high dose group. The low dose group and controls showed only a low incidence of desquamation. There was a dose-related decrease in mean group body weight of both males and females that became more pronounced over time; however, significance was reached only in the high dose females. Food consumption was also reduced but not to levels of statistical significance. All dosed rabbits showed statistically significant, dose-related increases in liver and kidney weight and organ to body weight ratios. Diffuse hepato-cytomegaly was evident in livers from 9/10 high dose and 4/10 low dose rabbits; effects ranged from slight to severe. Cytoplasmic degeneration and vacuolated hepatocytes were occasionally seen. Thymic atrophy was observed in 6/8 high dose rabbits but not in the low dose group. No other microscopic abnormalities were observed. Blood cholesterol levels were significantly elevated in a dose related manner in both sexes; other clinical chemistry values were within normal ranges. |
| <u>Conclusions</u> (study authors) | The test substance elicited dermal irritation as well as systemic effects that might have been related to weight loss or stress. Hepatic alterations were manifested as liver enlargement, |

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| <p><u>Quality</u></p> <p>Reliabilities</p> <p><u>References</u></p> <p><u>Other</u></p> <p>Last changed</p> | <p>elevated serum cholesterol, gross observations of liver abnormalities, and microscopic findings of diffuse hepatocytomegaly, cytoplasmic degeneration and hepatocellular vacuolization. Kidney weight and kidney weight to body weight ratio were elevated in both sexes, in a dose responsive manner, but there were no histopathological abnormalities observed.</p> <p>1. Reliable without restrictions</p> <p>McKee, R.H., Kapp, R.W., and Ward, D.P. 1985. Evaluation of the systemic toxicity of coal liquefaction-derived materials following repeated dermal exposure in the rabbit. J. Appl. Toxicol. 6: 345-351.</p> <p>14-Dec-01 (Prepared by a contractor to the Olefins Panel).</p> |
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Robust Summary - Group 10: Coal-Derived Fuel Oils

Repeated Dose Toxicity

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| <u>Test Substance</u> | EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538 ⁰ C. |
| <u>Method</u> | Other. |
| Method/guideline followed | 90-Day oral toxicity. |
| Test type | Yes |
| GLP | 1984 (1987 publication) |
| Year | Rat |
| Species | Sprague-Dawley (Hilltop Laboratory Animals, Scottsdale, PA, 6 wks old at receipt) |
| Strain | Oral gavage. |
| Route of administration | Approx. 104 days |
| Duration of test | 0, 0.02, 0.1, 0.5 g/kg/day diluted in highly refined white oil (CAS #8012-95-1) |
| Doses/concentration levels | Male and female (18M, 18F/dose group) |
| Sex | 13 wks (90 days) |
| Exposure period | Once a day, 5 days/wk |
| Frequency of treatment | 36M, 18F; 5ml/kg white oil; once a day, 5 days/wk |
| Control group and treatment | 2 weeks. |
| Post exposure observation period | Bartlett's test of homogeneity of variance, standard ANOVA, Duncan's test, linear regression for dose response; Kruskal Wallis (non-parametric test) followed by Dunn's Summed Rank test if appropriate; Jonckheere's test for monotonic trends in dose groups. |
| Statistical methods | |
| Test Conditions | This assay was the subchronic toxicity portion of a combined Reproductive/Subchronic toxicity study with an initial group size of 18M, 54F/dose group and 36M, 90F/control. Animals were assigned to 4 treatment groups based on body weight and were housed individually in stainless steel cages in rooms maintained at 20-24 ⁰ C temperature, 40-70% relative humidity and 12 hr light/dark cycle. Animals received water and food ad lib. Test material was administered by gavage 5 times weekly for 13 wks; body weight was recorded prior to dosage initiation (day 0) and weekly throughout the exposure period. At the end of 13 wks of treatment, 18 female rats/group were selected for the subchronic study and the others mated. All males were mated for 10 days. Fourteen days after termination of dosing and following confirmation of mating, blood samples were collected from males and 18 females /group. Animals were killed and necropsied. Brain, kidneys, adrenals and reproductive organs were weighed and eleven organs as well as gross lesions and tissue masses, if any, were collected and processed for histopathology. High dose and control groups were examined microscopically. |
| <u>Results</u> | |
| NOAEL (NOEL) | NOAEL= 0.1g/kg , based on elevated liver weight and hematology effects at 0.5 g/kg (assigned by reviewer). |
| Remarks | No treatment related mortality or significant differences in food consumption or clinical signs, except for urogenital staining in high dose animals were observed. Body weight gain was significantly reduced in high dose males by 7% in wks 6-7 of dosing; wt gain in high dose females and all other treated animals was similar to controls. No apparent abnormalities in gross examination of visceral organs and no effects on organ weight of males in any group were observed. Absolute liver wt was elevated and brain wt was reduced in high dose females (p<0.05); brain wt was not significantly different from controls as a fraction of body weight. No treatment related microscopic changes in tissue from either sex in the high dose group were observed compared to controls. There were no gross lesions or masses. Erythrocyte counts, hemoglobin and hematocrit were significantly reduced in high dose females (p<0.01); hemoglobin was reduced in high dose males. Dose related changes in serum cholesterol (elevated) and SGOT levels (decreased) in high dose animals but fell within normal historical range of biological values. Other clinical chemistry parameters were not significantly different from concurrent vehicle control values. |

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| <p><u>Conclusions</u> (study authors)</p> <p><u>Quality</u> Reliability</p> <p><u>References</u></p> <p><u>Other</u> Last changed</p> | <p>Administration of EDS coal-derived experimental fuel oil at doses up to 0.5g/kg/day for 13 weeks induced slight systemic toxicity in male and female rats, including reduced body wt gain, elevated liver weight, reduced hematology values, and elevated cholesterol which are qualitatively consistent with results of repeat exposure studies of other coal-derived liquids of similar boiling range.</p> <p>2. Reliable with restrictions. Analysis of dosing solutions was not performed for composition, purity or stability.</p> <p>McKee, R.H., Plutnick, R.T. and Traul, K.A. 1987. Assessment of the potential reproductive and subchronic toxicity of EDS coal liquids in Sprague Dawley rats. Toxicology 46: 267-289 (See separate summary for reproductive study). McKee, R.H. et al J. Appl. Toxicol. 4: 198-205 (additional analytical data on fuel oil)</p> <p>14-Dec-01 (Prepared by a contractor to the Olefins Panel).</p> |
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Robust Summary - Group 10: Coal-Derived Fuel Oils

Toxicity to Reproduction

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| <u>Test Substance</u> | EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538 ⁰ C. |
| <u>Method</u> | Other. |
| Method/guideline followed | 1-generation reproductive toxicity. |
| Test type | Yes. |
| GLP | 1985 (1987 publication). |
| Year | Rat. |
| Species | Sprague-Dawley (Hilltop Laboratory Animals, Scottdale, PA, 6 wks old at receipt). |
| Strain | Oral gavage. |
| Route of administration | Approx. 142 days (90 days exposure, 10 days mating, 40-42 days gestation and lactation). |
| Duration of test | 0, 0.02, 0.1, 0.5 g/kg/day diluted in highly refined white oil (CAS #8012-95-1). |
| Concentration levels | Male and female (18M, 36F/dose group). |
| Sex | 13 weeks (90 days) |
| Exposure period | once a day, 5 days/wk |
| Frequency of treatment | 36M, 72F; 5ml/kg white oil; once a day, 5 days/wk |
| Control group and treatment | |
| Statistical methods | Bartlett's test of homogeneity of variance, standard ANOVA, Duncan's test, linear regression for dose response; Kruskal-Wallis (non-parametric test) followed by Dunn's Summed Rank test if appropriate; Jonckheeres test for monotonic trends in dose groups. Standard nested ANOVA for fetal body parameters by sex nested within dam and dam within dose group analysis followed by least significant differences technique. |
| Remarks for Test Conditions. | This assay was the reproductive portion of a combined Reproductive/Subchronic toxicity study with an initial group size of 18M, 54F/dose group and 36M, 90F/control. Animals were assigned to 4 treatment groups based on body weight and were housed individually in stainless steel cages in rooms maintained at 20-24 ⁰ C temperature, 40-70% relative humidity and 12 hr light/dark cycle. Animals received water and food ad lib. Test material was administered by gavage 5 times weekly for 13 wks; body weight was recorded prior to dosage initiation (day 0) and weekly throughout the exposure period. At the end of 13 weeks of treatment, 18 female rats/group were selected for the subchronic study and the others mated. All males were mated, then killed and necropsied for the subchronic study. 18M, 36F/dose group; 36M, 72F/control group were housed together (1M:2F) for 10 consecutive nights or until mating was confirmed by vaginal plug or sperm in a vaginal rinse = Gestation Day (GD) 0. Mated females were maintained untreated throughout gestation and lactation (LD) to postpartum day 21. Maternal body weight was recorded on GD 0, 7, 14, 21, and LD 0, 7, 14, 21 and all dams were examined for viability, toxicological effects and unusual behavior. Offspring were examined grossly and weighed at LD 0, 4, 14, 21. All dams and surviving litters were killed and grossly examined on LD21. Pups were necropsied and visceral organs and brain examined. Pups that died spontaneously were also necropsied unless precluded by autolysis or cannibalism. |
| <u>Results</u> | |
| NOAEL | NOAEL (maternal)= 0.5g/kg; NOAEL (offspring)=0.5g/kg (assigned by reviewer). |
| Remarks | No treatment related mortality or significant differences in body weight gain, food consumption or physical signs, except for urogenital staining in high dose animals were observed. Seven control animals, 1 low dose female, and 1 mid dose male were euthanized during the treatment period due to dosing injuries. Pregnancy indices were comparable in all dose groups: 80.9%, 86.1%, 88.9% and 83.3% in control, low, mid and high dose groups respectively. No significant differences were observed in length of gestation or in maternal weight gain during gestation. No treatment related effects were observed in mean litter size, live births or pup survival at LD 4, 14 and 21 or in pup body weight throughout lactation to weaning at LD21. Sex of pups was not reported. A low incidence of malformations occurred in all groups: Control: 4 pups in 4 litters – 2 tail abnormalities, 1 depressed |

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| <p><u>Conclusions</u> (study authors)</p> <p><u>Data Quality</u> Reliability</p> <p><u>References</u></p> <p><u>Other</u> Last changed</p> | <p>sternum, 1 short snout. Low dose: 4 pups in 2 litters – 2 syndactyly, renal agenesis, no sex organs (1 agnatha), 1 syndactyly and renal agenesis, 1 pup with small kidney. Mid dose: 2 pups in 2 litters – tail abnormalities. High dose: 4 pups in 2 litters – 1 agnatha, 2 tail abnormalities, 1 misshapen skull.</p> <p>Syndactyly and renal agenesis have been reported as a spontaneously occurring genetic syndrome in Sprague-Dawley rats (Schreiner et al., 1979). Also, in a parallel study on another coal liquid, 2 pups with this syndrome were reported in 2 control litters.</p> <p>Coal-derived experimental fuel oil administered orally to male and female rats at doses up to 0.5g/kg for 13 weeks prior to mating did not adversely affect reproductive capacity or performance. There was no treatment-induced effect on overall incidence of abnormalities in any treated group or increased incidence of any specific class of malformations.</p> <p>2. Reliable with restrictions. Analysis of dosing solution was not performed for composition, purity or stability.</p> <p>McKee, R.H., Plutnick, R.T. and Traul, K.A. 1987. Assessment of the potential reproductive and subchronic toxicity of EDS coal liquids in Sprague Dawley rats. Toxicology 46: 267-289 (See separate summary for subchronic study). McKee, R.H. et al J. Appl. Toxicol. 4: 198-205 (additional analytical data on fuel oil) Schreiner, C.A. et al. 1979. Teratology 19: 46(A).</p> <p>14-Dec-01 (Prepared by a contractor to the Olefins Panel).</p> |
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Robust Summary - Group 10: Coal-Derived Fuel Oils

Developmental Toxicity

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| <p><u>Test Substance</u></p> | <p>EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538°C.</p> |
| <p><u>Method</u></p> <p>Method/guideline followed Type of Study GLP Year Species/Strain Sex Number/sex/dose Route of administration Exposure Period Concentrations Controls Statistical methods</p> | <p>OECD 414 Developmental toxicity. Yes. 1984. Sprague-Dawley Rats. Pregnant females. 25 females/dose. Oral gavage. Days 6-19 of gestation. 0, 0.1, 0.5, or 1.0 g/kg diluted in highly refined white oil at 5 ml/kg volume (CAS #8012-95-1). Controls received 5 ml/kg of white oil. ANOVA, Bartlett's Test, Kruskal-Wallis non-parametric test, Fisher's Exact Test.</p> |
| <p>Remarks for Test Conditions</p> | <p>Physical examinations were performed and body weights were measured on gestational days 0, 6, 10, 15, and 20. Mated females were sacrificed on gestational day 20 and a gross necropsy was performed. Uteri and ovaries were weighed and corpora lutea were counted. The number of implantation sites, early and late resorptions, and live and dead fetuses were determined. Full term fetuses were examined for abnormalities, weight, and crown-rump distance. From each litter, the heads of half of the fetuses were preserved and examined, while the other half of the fetuses were examined for skeletal malformations and ossification variations.</p> |
| <p><u>Results</u></p> | <p>NOAEL maternal: 0.1 g/kg. NOAEL fetal: 0.1 g/kg.</p> |
| <p>Remarks</p> | <p><u>Maternal:</u> Maternal body weight gain (GD 6-20) and uterine weight at term were significantly reduced in the mid and high dose groups. There also were increased clinical observations in these groups that included nasal, ocular, oral, and vaginal discharges, rales, and ungroomed appearance.</p> <p><u>Fetal:</u> The mid and high dose groups exhibited significant increases in early embryonic resorptions with corresponding decreases in the mean number of live fetuses (86% and 25% compared to 100% in the control and low dose). The remaining fetuses in the mid and high dose group had significantly reduced fetal body weight and crown-rump distance. The overall number of fetal skeletal malformations were not significantly different from the controls, although the ratios of malformed fetuses per litter were significantly increased in the mid and high groups. Gross visceral abnormalities were observed only in the mid and high dose groups.</p> |
| <p><u>Conclusions</u> (submitter)</p> | <p>Coal-derived experimental fuel oil administered orally to pregnant female rats was embryo-lethal and teratogenic in rats at doses that are maternally toxic. Under the conditions of this study, the test substance was not a selective developmental toxicant.</p> |
| <p><u>Data Quality</u> Reliability</p> | <p>2. Reliable with restrictions. Analysis of dosing solution for composition, purity or stability. was not performed</p> |
| <p><u>Reference</u></p> | <p>Exxon Biomedical Sciences (1984) "Oral teratology study in rats," Unpublished study.</p> |
| <p><u>Date last changed</u></p> | <p>14-Dec-01 (Prepared by a contractor to the Olefins Panel).</p> |

Robust Summary - Group 10: Fuel Oils

Acute Toxicity

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| <u>Test Substance</u> | Light Pyrolysis Fuel Oil, CAS #68527-18-4. Water-white liquid. Refer to sponsor for purity and stability. |
| <u>Method</u> | |
| Method/guideline followed | Standard method (not referenced) with doses based on a previous limit test and two range-finding studies. |
| Type (test type) | Acute LD ₅₀ |
| GLP | Yes |
| Year | 1984 |
| Species/Strain | Rat, Fischer 344 |
| Sex | Male and female |
| No. of animals per sex/dose | 5 |
| Vehicle | None |
| Route of administration | Oral |
| Test Conditions | Rats (74 days old, 113.4-201.2g) were individually housed in screen-bottomed cages in a room with 71.3°F temperature, relative humidity of 46.4% and 12 hr light/dark cycle. Chow diet and water were available ad lib. Rats were fasted for 24 hrs prior to dosing at 2.50, 2.75, 3.00 and 3.25g/kg. Rats were observed at 1 and 4 hrs after oral gavage dosing on day 1, and at least once daily thereafter with exception of days 5, 6, 7, 13 and 14 (holidays and week-ends). Observations for mortality and moribundity were performed daily until sacrifice on day 15. Gross necropsies were performed on all rats. Oral LD ₅₀ were calculated by Probit analysis. |
| <u>Results</u> | |
| LC ₅₀ with confidence limits. | LD ₅₀ combined sexes (95% confidence limits) = 2.89g/kg (2.63-3.28g/kg). Females were somewhat more susceptible than males. No effects seen on body wt. The most frequently observed clinical signs were nasal and ocular discharges, lethargy, and soft stools. Gross necropsies revealed no adverse findings. |
| Remarks | |
| <u>Conclusions</u> | |
| (study author) | LD ₅₀ combined sexes (95% confidence limits) = 2.89g/kg (2.63-3.28g/kg). |
| <u>Data Quality</u> | |
| Reliability | 1. Reliable without restrictions |
| <u>References</u> | Rausina, G.A., 1984. Acute oral toxicity study in rats of Light Pyrolysis Fuel Oil. Proj. #2101. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX |
| <u>Other</u> | |
| Last changed | Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary - Group 10: Fuel Oils

Acute Toxicity

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| <u>Test Substance</u> | Light Pyrolysis Fuel Oil, CAS # 68527-18-4. Clear greenish-blue liquid. Refer to sponsor for purity and stability. |
| <u>Method</u> | |
| Method/guideline followed | Standard method (not referenced) |
| Type (test type) | Limit test |
| GLP | Yes |
| Year | 1984 |
| Species/Strain | Rat, Fischer 344 |
| Sex | Male and female |
| No. of animals per sex/dose | 5 |
| Vehicle | None |
| Route of administration | Inhalation (whole body exposure) |
| Test Conditions | Rats (11 wks old, 100-172g) were individually housed in stainless steel, screen-bottomed cages in a room maintained at 72.9°F, relative humidity of 51% and 12 hr light/dark cycle. Rats received chow diet and water ad lib, except during exposure. One group of 10 rats was exposed to aerosolized test article, generated by a ball jet nebulizer, for 4 hrs. A condensing flask was used to prevent large particles from entering the chamber. Actual chamber concentration was 4.95g/m ³ (range 3.89-5.89g/m ³) as determined by gas chromatography. Median aerodynamic diameter was 3.2 microns. Rats were observed for clinical signs immediately after exposure, at 1 and 4 hrs post-dose, and daily thereafter over 14 days. Observations for mortality and moribundity were made twice on weekdays and once on weekends. Body wt was determined prior to exposure on day 1, on day 8, and before sacrifice. Gross necropsies were performed on day 15. |
| <u>Results</u> | |
| LC ₅₀ with confidence limits. | LC ₅₀ was not reached at 4.95g/m ³ . There were no deaths during the study, and no gross pathological changes were seen at necropsy. Immediately after exposure, all rats exhibited hair discoloration, perianal soiling, dry red material around nose/mouth, and rales. Incidences of rales and/or sneezing/wheezing were sporadically seen in both sexes until day 14. Nasal and ocular discharge, red nose, ocular porphyrin, and partial closing of the eyes were also evident during the post-exposure period. There was initial body wt loss in both sexes by day 8, with recovery to initial wt or above by day 15. |
| Remarks | |
| <u>Conclusions</u> (study author) | No deaths were observed during the study; therefore the LC ₅₀ was not reached at 4.95g/m ³ . Major clinical signs included hair discoloration, nasal discharge, rales, and dry red material around nose/mouth |
| <u>Data Quality</u> | |
| Reliability | 1. Reliable without restrictions |
| <u>References</u> | Rausina, G.A., 1984. Acute inhalation toxicity study in rats of Light Pyrolysis Fuel Oil. Proj. #2102. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX |
| <u>Other</u> | |
| Last changed | Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary - Group 10: Fuel Oils

Genetic Toxicity - in Vitro

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| <u>Test Substance</u> <i>Test substance</i> | Light Pyrolysis Fuel Oil, Gulf. CAS #68527-18-4. Water-white liquid. Composition analysis, purity and stability referred to sponsor. |
| <u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested | Standard method based on Hsie et al. (1981), O'Neill & Hsie (1979) In vitro mammalian cell forward mutation Chinese hamster ovary (CHO) cell culture Yes 1985 CHO-K-1 heterozygous for hypoxanthine-guanine phosphoribosyl transferase (HGPRT+/-) from Oak Ridge National Laboratory, TN. Yes Rat liver (S9) fraction purchased from Litron Labs, Rochester, NY 1.0mg S9 fraction/ml treatment medium in range finding trial; 0.5mg S9 fraction/ml medium in mutagenicity test Aroclor 1254 induced (treatment not specified) Range finding (Cytotoxicity): 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 5000µg/ml ± S9; Mutagenicity test #1: (8, 16 cytotoxicity only), 25, 32, 50, 64, 128µg/ml ±S9; repeat +S9: 50, 64µg/ml, all diluted in 50% Pluronic [®] polyol F127 (prepared in absolute ethanol, mol. wt. 12,500). |
| Statistical Methods | Frequency of mutant colonies per million clonable cells, corrected for absolute survival by viability plates, was calculated and comparisons of treated cultures with vehicle controls made on transformed data using a two-tailed t-test (Irr & Snee, 1979) using the MUTANT computer program (Snee et al., 1981). Criteria for positive results were significant (p<0.05) increase in mutant colonies (HGPRT+/- ? HGPRT -/-) at any dose level and a dose related response. If only one criterion is met, results are considered equivocal. |
| Remarks for Test Conditions | Sufficient Light Pyrolysis Fuel Oil (LPFO) was weighed separately for each dose level into 1ml volumetric flasks and stored overnight. The following day, 0.18ml of 50% F127 was added to each flask along with sufficient medium (Ham's F-12 without hypoxanthine) to achieve final 1ml volume for testing. All dosing preparations were vortexed just after addition of medium and just prior to use. All cultures were incubated at 37°C in 5% CO ₂ enriched, humidified atmosphere. Positive control mutagens were ethyl methanesulfonate (100µg/ml) for -S9 cultures, and benzo(a)pyrene (4µg/ml) for +S9 cultures. For range finding (cytotoxicity), each dose group was composed of 2 flasks, one -S9, one+S9, negative controls ± S9, seeded with 5x10 ⁵ cells on day 1. Cultures were exposed to test compound for 5 hours on day 2. On day 3, cells were trypsinized and counted with a Coulter Model ZB, then 200 cells were transferred into each of 3 60mm culture dishes. These viability plates were incubated until day 10, fixed in methanol and stained with Giemsa. Colonies were counted visually or with an Artek Model 981 colony counter. Absolute survival = total colony count ÷ number of cells seeded/flask. Relative survival = absolute survival in treated cultures ÷ vehicle control survival. Acceptable survival level is at least 10%. An S9 concentration test using both viability and mutagenicity plates was performed at doses of 16 or 50µg/ml LPFO and S9 concentrations of 0.25, 0.5, 1.0mg/ml to select optimal S9 concentration for the definitive mutagenicity test. For mutagenicity, cells were seeded on day 1 into 6 flasks/dose group, 3-S9, 3+S9; on day 2 approximately 10 ⁶ cells were exposed to LPFO for 5 hours. Vehicle control had 12 flasks, 6-S9, 6+S9. On day 3, cultures with excessive cytotoxicity were discarded. From remaining cultures, 200 cells were seeded to each of 4 viability plates/dose level; incubated to day 10, fixed with methanol, stained with Giemsa, and colonies counted for survival. Expression cultures (10 ⁵ -10 ⁶ cells/one dish/dose) were seeded on day 3; subcultured three times until day 10 when 200 cells were seeded on each of 4 viability plates/dose and 2x10 ⁵ cells seeded on each of 5 mutagenicity plates/dose with selective medium containing 10 ⁻⁵ M 6-thioguanine to allow expression of HGPRT mutation. Cultures were incubated undisturbed until day 17 |

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| | <p>when they were fixed and stained. For mutagenicity, a ratio of total colony counts in mutagenicity plates over absolute survival in viability plates was calculated for each treatment group. Frequency of mutant colonies/million clonable cells was calculated and statistical comparisons with negative control data were made. A repeat mutagenicity test with metabolic activation at two doses was also performed</p> |
| <p><u>Results</u> Genotoxic effects</p> | <p>In the range finding test, LPFO induced post treatment cell toxicity at all dose levels with and without S9 (cells $\times 10^5$/ml: -S9 5.3, 3.5, 3.7, 3.3 and 0.0 at 0[vehicle control], 8, 16, 32, and 64μg/ml; +S9 3.4, 3.0, 2.9, 1.1, 0.57 and 0.3 at 0[vehicle] 16, 32, 64, 128 and 256μg/ml). No reduction in colony counts were observed in any non-activated dose groups with sufficient cells to clone (8, 16, 32μg/ml); in S9 activated cultures, significant cloning toxicity occurred at 64 and 128μg/ml (Relative survival 4.1 and 19.5%). The S9 concentration selected for the definitive mutagenicity test was 0.5mg/ml based on the greatest number of mutant colonies observed at this concentration in cultures treated with 50μg/ml LPFO. In the mutagenicity test, cell toxicity occurred at LPFO doses of 32μg/ml and higher in -S9 cultures (cells $\times 10^5$/ml 5.0, 4.2, 2.4 and 0.67 at 0[vehicle], 32, 50 and 64μg/ml, respectively) and at 16μg/ml and higher doses in +S9 cultures (cells $\times 10^5$/ml 4.8, 4.2, 2.2, 2.3, 2.4 and 1.1 at 0[vehicle], 16, 32, 50, 64 and 128μg/ml, respectively). Cultures treated with 8 and 16μg/ml \pm S9 were not subcultured for mutagenicity. Cloning efficiency decreased in +S9 cultures at all doses (Relative survival: 9.0, 2.5, 8.4 and 1.3% at 32, 50 64 and 128μg/ml, respectively). No significant increase in mutant colonies and no dose related response was observed in any culture - S9 treated with LPFO. Positive control compounds demonstrated appropriate responses (EMS-S9 118.1 mutants/10^6 clonable cells; B(a)P 198.1 mutants/10^6 clonable cells). Due to an unexpected high value in one outlier culture flask (340.2 mutants) in the 64μg/ml +S9 dose group, a repeat activated test was performed at does of 50 and 64μg/ml. No significant increases in number of mutant colonies or a dose response were observed in the repeat assay (24.8, 7.9 and 1.4 mutants/10^6 cells at 0[vehicle], 50 and 64μg/ml).</p> |
| <p><u>Conclusions</u> (contractor)</p> | <p>Light Pyrolysis Fuel Oil did not induce a mutagenic response with or without metabolic activation in CHO/HGPRT cells at any dose level. Light Pyrolysis Fuel Oil does not cause gene point mutations under the conditions of this assay.</p> |
| <p><u>Data Quality</u> Reliabilities</p> | <p>1. Reliable without restrictions.</p> |
| <p><u>Reference</u></p> | <p>Papciak, R.J. 1985. CHO/HGPRT test of Light Pyrolysis Fuel Oil. Proj. #2105. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX Hsie, A.W. et al. 1981. Mut. Res. 86: 193-214 O'Neill, J.P. and Hsie, A.W. 1979. Banbury Report 2: 55-63 Irr, J.D. and Snee, R.D. 1979. Banbury Report 2: 263-275. Snee, R.D., Smith, R.L., and Irr, J.D. 1981. MUTANT. A computer program for the evaluation of short-term mutation test results. E.I. Dupont de Nemours Co.</p> |
| <p><u>Other</u> Last changed</p> | <p>Revised 9/25/2001 (Prepared by a contractor to the Olefins Panel)</p> |

Robust Summary - Group 10: Fuel Oils

Genetic Toxicity - in Vitro

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| <u>Test Substance</u> <i>Test substance</i> | Light Pyrolysis Fuel Oil, Gulf. CAS #68527-18-4. water white liquid. Composition analysis, purity and stability referred to sponsor. |
| <u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested Exposure period Statistical Methods | Standard method based on Williams et al. (1977,1982) In vitro mammalian cell DNA repair assay Unscheduled DNA Synthesis (UDS) in primary hepatocyte cultures. Yes 1984 Fischer 344 male rat (8 wks old) No NA NA NA 8, 16, 32, 64, 128, 256, 512, 1024 µg/ml, diluted in 50% Pluronic [®] polyol F127 (prepared in absolute ethanol, mol. wt 12,000, 70% hydrophilic) 18 hours None specified. Criteria for positive response are incorporation of radioactive precursor (³ H-thymidine) in cells that are not normally synthesizing DNA, indicating repair of damage. A positive response is defined as a mean net nuclear grain count at any treatment level that exceeds concurrent negative control by at least 6 grains/nucleus; negative control value must not exceed 5 grains. If this criterion is not met, a positive response can be identified if there is a significant difference (p<0.01) in % cells in repair at any dose level and negative control value. This indicator defines whether a small fraction of cells is undergoing repair (Casciano & Gaylor, 1983). A positive response need not be dose related. |
| Remarks for Test Conditions | Sufficient Light Pyrolysis Fuel Oil (LPFO) was weighed separately for each dose level, 0.46ml of 50% F127 added per ml of final volume and sufficient medium (Williams Medium E with 10% fetal bovine serum and insulin) added to achieve final volume. Test preparations were mixed just prior to addition at 30µl to each 3ml culture. The conc. of ³ H-thymidine (½ life 12.5 yrs.) used in these assays was 1mCi/ml. All cultures were incubated at 37°C in 5% CO ₂ enriched humidified atmosphere. No range finding assay was performed. In the UDS assay, 2x10 ⁵ cells/ml were seeded into coverslip cultures, exposed to ³ H-thymidine and test substance for 18 hours (3 cultures/dose level, 8 dose levels), untreated controls, vehicle F127control and positive control, 2-acetyl aminofluorene (0.01µg/ml). Cells growing on coverslips were rinsed, fixed and glued to microscope slides on day 2. On day 3, slides were dipped in autoradiographic emulsion and stored in the dark at 2-8°C. Technical error required the emulsion step be repeated on day 15; first emulsion was peeled off and slides were dipped in fresh emulsion. Autoradiographs were developed, stained and coverslipped on day 22. Numbers of grains overlying 50 randomly selected nuclei/slide were counted. The highest of 3 cytoplasmic grain counts/cell were subtracted and this number was divided by a conversion factor of 2 to obtain net nuclear grain count. Avg. net nuclear grain count/slide (sum of net nuclear grain count ÷ 50) and mean net nuclear grain count (avg. net nuclear grain count/slide ÷ 3) were calculated. In addition, % cells in repair was determined for each dose level. |
| <u>Results</u> Genotoxic effects | LPFO induced significant toxicity in primary hepatocytes following 18 hours exposure, leaving too few cells for UDS analysis at doses = 64µg/ml. LPFO caused dose related UDS at all non-toxic levels. Percentage of cells in repair increased from the vehicle control value of 2.7% to 43.3% at 8µg/ml to 96.0% at 32µg/ml. Statistics were not applied because LPFO induced avg. net nuclear counts >6 grains above neg. controls at all non-toxic levels. Positive and negative controls gave expected responses. |
| <u>Conclusions</u> (contractor) | Light Pyrolysis Fuel Oil induced dose related unscheduled DNA synthesis in primary cultures of rat hepatocytes beginning at the lowest dose tested. Light Pyrolysis Fuel Oil |

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| <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p> <p><u>Other</u> <i>Last changed</i></p> | <p>cultures of rat hepatocytes beginning at the lowest dose tested. Light Pyrolysis Fuel Oil causes DNA damage and repair under conditions of this assay.</p> <p>2. Reliable with restrictions. Limited specific data supplied. Statistical criteria described but statistical method not cited or employed</p> <p>Brecher, S. 1984. Hepatocyte primary culture/DNA repair test of light pyrolysis fuel oil. Proj. #2107. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX</p> <p>Williams, G.M. 1977. Cancer Res. 37: 1845-1851</p> <p>Williams et al. 1977. In Vitro 13: 809-817</p> <p>Williams et al. 1982. Mut. Res. 97:359-370</p> <p>Casciano, D.A. and Gaylor, D.W. 1983. Mut. Res. 122:81-86</p> <p>4/11/2001 (Prepared by a contractor to the Olefins Panel)</p> |
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Robust Summary - Group 10: Fuel Oils

Genetic Toxicity - in Vitro

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| <u>Test Substance</u> <i>Test substance</i> | Light Pyrolysis Fuel Oil, Gulf. CAS #68527-18-4. Water-white liquid. Composition analysis, purity and stability referred to sponsor. |
| <u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested | Standard method based on Cortesi et al (1983), Dunkel et al (1981), Reznikoff et al (1973) In vitro cell transformation Mouse embryo cells Yes 1984 BALB/3T3-A31 -1-1 from T. Kakunaga, National Cancer Inst., 1982 No NA NA NA Cytotoxicity: 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 5000µg/ml; Transformation: 20, 60, 90, 110µg/ml, all diluted in 50% Pluronic [®] polyol F127 (prepared in absolute ethanol, mol. wt. 12,000, 70% hydrophilic). |
| Exposure period Statistical Methods | 2 days None employed. Criteria for positive response were a two-fold increase in type III foci at the highest dose over vehicle control (at least 2 type III foci if vehicle control had none) with or without a dose related response, or a two-fold increase at two or more consecutive doses. Test is equivocal if two-fold increase occurred at any one level other than the highest acceptable dose. |
| Remarks for Test Conditions | Sufficient Light Pyrolysis Fuel Oil (LPFO) was weighed separately for each dose level, 0.46ml of 50% F127 added per ml of final volume and medium(Eagles MEM with 10% heat-inactivated fetal calf serum) added as required to achieve final volume for testing. Test preparations were mixed just prior to addition to cultures at 50µl to each 5 ml culture. All cultures were incubated at 37 ⁰ C in 5% CO ₂ enriched humidified atmosphere. For cytotoxicity, 2 cultures/dose group, 2 cultures for vehicle F127 or medium negative control were seeded with 1x10 ⁴ cells/culture in day 1, exposed on days 2-3, trypsinized and counted with a Coulter Model ZB on day 4 for at least 10% survival. For transformation, 15 cultures (1x10 ⁴ cells/culture/dose group)) and two colony formation cultures (100 cells/culture/dose group) were seeded on day 1, exposed on days 2-3 and culture medium changed on day 4. For transformation cultures, medium continued to be changed weekly to day 29. Positive control was 3-methylcholanthrene (1µg/ml). Colony formation cultures were fixed, stained, and counted visually on day 9 to determine cloning efficiency (avg. number colonies/plate ÷ 100 cells seeded). Transformation cultures were fixed and stained on day 29 for focus counting and evaluation. Transformation frequency = total type III foci ÷ total flasks/dose group. |
| <u>Results</u> Genotoxic effects | LPFO induced toxicity in BALB/3T3 cells after two days exposure beginning at 32µg/ml (74.8% relative survival); viability dropped sharply at 128µg/ml (6% relative survival) and was 100% toxic at higher concentrations. LPFO did not induce transformed foci in excess of negative control cultures at any dose level. Toxicity was evident at 60µg/ml (10% relative cloning efficiency). Positive and negative controls gave expected responses. |
| <u>Conclusions</u> (contractor) | Light Pyrolysis Fuel Oil did not induce transformation in BALB/3T3 cells at any dose level under conditions of this assay. |
| <u>Data Quality</u> Reliabilities | 1. Reliable without restriction. |
| <u>Reference</u> | Brecher, S. 1984. Cell transformation test of Light Pyrolysis Fuel Oil. Proj. #2108. Gulf |

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| <p><u>Other</u> <i>Last changed</i></p> | <p>Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co, Houston, TX Cortesi, E. et al. 1983. Teratogenesis, Carcinogenesis, Mutagenesis 3: 101-110. Dunkel, V.A. et al. 1981. J. Nat'l Cancer Inst. 67: 1303-1315. Reznikoff, C.A. et al. 1973. Cancer Res. 3239-3249.</p> <p>7/27/2001 (prepared by a contractor to the Olefins Panel).</p> |
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Robust Summary - Group 10: Fuel Oils

Genetic Toxicity - in Vivo

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| <u>Test Substance</u> | Light Pyrolysis fuel oil, Gulf CAS #68527-18-4 Water white liquid. Compositional analysis, purity and stability referred to sponsor. |
| Remarks | |
| <u>Method</u> | |
| Method/guideline followed | Comparable to standard assay |
| Type | Mammalian bone marrow erythrocyte micronucleus |
| GLP | Yes |
| Year | 1984 |
| Species | Mouse |
| Strain | Crl:CD [®] -1 (ICR) BR Swiss |
| Sex | Male and female: Range finding (RF) 2M, 2F/group; Micronucleus 10M, 10F/group; 15M, 15 F in 1 group (11-12wks old at study initiation). |
| Route of administration | Oral gavage |
| Doses/concentration levels | RF:0, 1.25, 2.5, 5.0 g/kg in corn oil; Micronucleus: 0, 0.25, 0.5, 1.0 g/kg |
| Exposure period | 1 dose/day for 2 days; 1 group- 1 dose, 1 day only |
| Statistical methods | Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes (PCEs) with micronuclei (MN) , and group mean ratios of PCE to normochromatic erythrocytes (NORMs) were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant (p<0.05) increases in micronucleated PCE at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met. |
| Remarks for Test Conditions. | Light pyrolysis fuel oil (LPFO) dosing solutions were prepared fresh for each day of dosing – for RF 2.5 g LPFO mixed with corn oil to make 10 ml; for micronucleus 2.5 g LPFO mixed with corn oil to make 50 ml, blended by shaking. Based on results of range finding study, three groups of mice were given LPFO by oral gavage daily for two days. All mice were weighed on day 1 and on day of sacrifice. One half of each treated group and vehicle control (5M, 5F) was killed on day 3 and the remainder on day 4. One group (15M, 15F), given 1.0 g/kg by gavage in a single dose for 1 day only, was killed on days 2, 3, 4 (5/sex/day). Positive control mice given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Slides of femoral bone marrow smears were prepared, stained with May-Grunewald /Giemsa stain and examined microscopically. For each mouse, 1000 PCE and all associated mature erythrocytes (NORMs) were counted. Data collected included group mean body weights for each day, total PCEs, total NORMs, PCEs with MN, and NORMs with MN. |
| <u>Results</u> | |
| Genotoxic effects | In the range finding study, all mice given LPFO at 5.0 or 2.5 g/kg died by day 3. At 1.25 g/kg, ½ females died, surviving mouse was lethargic, males appeared normal. In micronucleus test, 1/10 males given 1.0 g/kg (2 doses) died by day 3. No other mortality or significant weight changes were observed. Surviving mice treated with LPFO did not show any significant changes in micronucleus formation in PCE and no significant changes in the ratio of PCE/NORM compared to vehicle controls.. |
| NOAEL (NOEL) | NOEL (genetic)= 1.0 g/kg. NOEL (systemic)= 0.5g/kg |
| LOAEL (LOEL) | |
| <u>Conclusions</u> | |
| (study authors) | Oral treatment of mice with Light Pyrolysis Fuel Oil for 1-2 days at doses up to 1.0 g/kg/day had no effect on frequency of micronucleated polychromatic erythrocytes in bone marrow. Under these test conditions LPFO does not induce cytogenetic damage. |
| <u>Data Quality</u> | |
| Reliabilities | 1. Reliable without restrictions. |
| <u>References</u> | |
| | Khan, S.H. 1984. Micronucleus test of Light Pyrolysis Fuel Oil. Proj. #2106. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX |
| <u>Other</u> | |
| Last changed | 7/27/2001 (Prepared by a consultant to the Olefins Panel) |

Robust Summary - Group 10: Fuel Oils

Repeated Dose Toxicity

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| <u>Test Substance</u> | Light Pyrolysis Fuel Oil, CAS# 68527-18-4 Water white liquid |
| Remarks | |
| <u>Method</u> | |
| Method/guideline followed | No guidelines specified, comparable to standard study. |
| Test type | Subacute |
| GLP | Yes |
| Year | 1985 |
| Species | Rat |
| Strain | F344 |
| Route of administration | Dermal |
| Duration of test | 8 days |
| Doses/concentration levels | 0, 1.0, 2.0 g/kg |
| Sex | Males and females |
| Exposure period | 6 hr/day |
| Frequency of treatment | once/day for 5 days |
| Control group and treatment | Light paraffinic oil (2.16 ml/kg) |
| Post exposure observation period | 2 days |
| Statistical methods | Analysis of Variance, Dunnett's test |
| Test Conditions | <p>Rats were housed in suspended stainless cages with wire mesh bottoms and fronts with automatic watering in a room maintained at 72.8°F, relative humidity of 56% and 12 hour light/dark cycle. Chow diet and water were provided ad lib. Doses were administered over 10% of body surface to the backs of rats clipped free of hair and fitted with Elizabethan collars to reduce ingestion. After 6 hours, collars were removed and residual oil wiped off. The test substance mixture was analyzed at initiation and termination of dosing period for concentration/stability/uniformity by fluorescence. Observations for mortality and moribundity were made daily, and for clinical signs daily on dosing days. Body weight was recorded at initiation and termination. Necropsy and gross pathology were conducted but histopathology, clinical pathology and organ weights were not obtained.</p> |
| <u>Results</u> | |
| NOAEL (NOEL) | NOEL not determined. |
| LOAEL (LOEL) | LOEL = 1 g/kg/day (based on skin irritation). |
| Remarks | <p>No mortality or morbidity was observed and there were no test article related clinical signs. By day 5, males and females in both dose groups had well defined erythema, with some resolution and eschar formation by day 8. Slight edema was seen in the high dose males and females that resolved by day 8. A significant decrease in body weight was noted in high dose males.</p> |
| <u>Conclusions</u> | <p>No mortality or morbidity were observed. Group mean body weight was decreased in males in the 2.0 g/kg group. Skin irritation occurred in rats receiving 1.0 and 2.0 g/kg of test article that partially resolved after the 2 day recovery period with severe erythema/eschar formation in 4 rats (3M, 1F) in the high dose group.</p> |
| <u>Quality</u> | |
| Reliabilities | 2. Reliable with restrictions. This study was limited in scope, being restricted to determination of morbidity/mortality, clinical signs including scoring for skin irritation, body weight changes and gross necropsy. |
| <u>References</u> | <p>Rausina, G. 1985. Five-day repeated dose dermal toxicity study in rats of light pyrolysis fuel oil. Proj. #2109A. Gulf Life Sciences Center, Pittsburgh, PA. For Gulf Oil Chemicals Co., Houston, TX</p> |
| <u>Other</u> | |
| Last changed | 2/15/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary - Group 10: Fuel Oils

Repeated Dose Toxicity

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| <p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Test type GLP Year Species Strain Route of administration Duration of test Doses/concentration levels Sex Exposure period Frequency of treatment Control group and treatment Post exposure observation period Statistical methods</p> <p>Test Conditions</p> <p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks</p> | <p>Light Pyrolysis Fuel Oil, CAS #68527-18-4. No analysis of purity or composition reported, referred to sponsor.</p> <p>No guidelines specified, comparable to standard study. Subacute Yes 1985 Rat Fischer 344 Whole body Inhalation 4 weeks 0, 0.51, 1.26, 2.54 g/m³ (actual) Males and females (10/sex/group) 6 hr./ day 5 days/wk. For 4 wks. Filtered air, 6 hrs/day, 5 days/wk. None Analysis of variance, Dunnett's test, Kolmogorov-Smirnov test</p> <p>Rats were housed individually in stainless steel, screen-bottomed cages with automatic watering systems in rooms maintained at 72.7°F with 59% relative humidity and a 12 hour light/dark cycle. Chow diet and water were provided ad lib except during exposure. Chamber concentrations were monitored by GC; peak areas were compared with those of liquid test article standards. Target dose levels were 0.5, 1.25, and 2.5 g/m³ administered as an aerosol. (Actual levels cited above). Each rat was observed twice daily on weekdays and once daily on weekends for morbidity and mortality, and once daily (immediately after exposure) for clinical signs. Body weight was taken at initiation and weekly thereafter. Blood was taken from non-fasted rats via the orbital sinus. At study termination, rats were necropsied for gross lesions and organs/tissues (34/rat) were preserved for histopathological evaluation (0, 1.26 and 2.54 g/m³ groups).</p> <p>Not determined LOEL = 0.51 g/m³ (decreased. body wt, dec. hematocrit, hemoglobin and blood glucose) After 4 exposures to 2.5 g/m³, 75% of the rats were found dead or moribund; remaining rats were sacrificed and only histopathological evaluations were performed on these rats. After the last exposure, 2 females at the 1.25 g/mg³ were sacrificed moribund. No other mortality was observed. The most frequent clinical signs in all dose groups were ocular porphyrin and discharge, closing of the eyes and nasal discharge; the highest incidence was in the 2.5 g/m³ group. Significant decreases in body weight were present in all test article exposed groups, and, with the exception of the spleen, were associated with consistent increases in organ to body wt. ratios. Both clinical signs and body weight changes were correlated with dose. Total white blood cell and platelet counts were elevated at 1.26 g/m³, and occasionally at 0.51 g/m³; decreased mean corpuscular hemoglobin was seen in females of both these dose groups, and in males at 0.51 g/m³. White cell increases were attributed to increased numbers of segmented neutrophils. Blood glucose levels were increased in males and females at 2.54 g/m³ and in males at 1.26 g/m³. Gross pathological effects included alopecia, perianal soiling, abnormal coloration of liver, lack of body fat. There was a variety of histopathological findings with the most severe being necrosis of cortical thymus lymphocytes especially in the high dose females. Atrophy of the thymic cortex was seen in 1.26 and 2.54 g/m³ males and females. Changes in the thymus were accompanied by atrophy of splenic lymphoid tissue, especially in high dose females, lymphoid hyperplasia in lungs, and hypoplasia of bone marrow. Hyaline droplets were observed in male kidneys in the 1.26 g/m³ and 2.54 g/m³ groups. Generalized vascular congestion was seen in both</p> |
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| <p><u>Conclusions</u> (study authors)</p> <p><u>Quality</u> Reliabilities</p> <p><u>References</u></p> <p><u>Other</u> Last changed</p> | <p>sexes in the 1.26 g/m³ and 2.54 g/m³ groups which was particularly prominent in bone marrow, kidney, adrenal, lung and thymus.</p> <p>No mortality or moribundity was seen in the 0.54 g/m³ group. Significant decreases were noted in mean body weight of both sexes in all groups receiving test material. Treatment was associated with significant histopathological effects including severe atrophy of the thymic cortex, hypoplasia of bone marrow and hyaline droplet degeneration of male kidney tubular epithelium. These changes were related to test material dose.</p> <p>1. Reliable without restrictions.</p> <p>Rausina, G. 1985. Four-week repeated dose inhalation toxicity study in rats of Light Pyrolysis Fuel Oil. Proj. #84-2111. Gulf Life Sciences Center, Pittsburgh, PA. For Gulf Oil Chemicals Co., Houston, TX</p> <p>2/20/2001 (Prepared by a contractor to the Olefins Panel)</p> |
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Robust Summary - Group 10: Fuel Oils

Repeated Dose Toxicity

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| <u>Test Substance</u> | Pyrolysis Fuel Oil, (water and oil quenched). Tested as supplied by producer. Produced by pyrolysis at temperatures up to 950 ⁰ C, contained 300-500 ppm benzo(a)pyrene |
| Remarks | |
| <u>Method</u> | |
| Method/guideline followed | No guidelines specified |
| Test type | Chronic/cancer study – skin painting |
| GLP | No |
| Year | 1977 |
| Species | Mouse (40/group/dose) |
| Strain | C3H/HeJ |
| Route of administration | Dermal |
| Duration of test | Lifetime (28 mon) |
| Doses/concentration levels | One brushfull – Mon, Wed., Fri. |
| Sex | Not specified |
| Exposure period | Continuous, no test article removal |
| Frequency of treatment | 3 times/wk. |
| Control group and treatment | Water- 1 brushfull 3 times/wk |
| Post exposure observation period | None |
| Statistical methods | Least squares to determine median latent period |
| Test Conditions | Doses of neat test article or water were brushed on to the backs of mice, clipped free of hair, three times/wk. Doses were applied consistently to the midline but qualitatively, with each dose described as a brushfull. At monthly intervals, papilloma or carcinoma indexes were calculated (100 times the ratio of number of mice with skin tumors divided by the effective group). Effective group was calculated as original number of mice at start divided by the number dead without tumors, but maintained constant after appearance of the median tumor. Median papilloma or carcinoma latent periods were also determined. Lesions were verified by histopathology. |
| <u>Results</u> | |
| NOAEL (NOEL) | Not applicable |
| LOAEL (LOEL) | Not applicable |
| Remarks | Results of the water-quenched and oil-quenched oils were essentially identical. Both samples were highly carcinogenic. For the water quenched oil, the papilloma and carcinoma indexes were 100 and 97.2, respectively, and the median papilloma and carcinoma latent periods were 10.2 and 12.2 months, respectively. For the oil quenched oils, the indexes were 94.4 and 94.4, respectively, and the latent periods 10.3 and 12.1 months. The malignant tumors were squamous cell carcinomas. |
| <u>Conclusions</u> (study authors) | Pyrolysis fuel oil (both water and oil quenched) was carcinogenic in the mouse skin painting bioassay. |
| <u>Quality</u> | |
| Reliabilities | Reliable with restrictions. Although the method used the early qualitative procedure of actual skin painting rather than exact volume application, and the material was not analyzed, the results are unambiguous. The described process conditions and high B(a)P levels are consistent with a dermal carcinogenic response and the latency period was short enough to anticipate possible metastatic spread (Reviewer's comments). |
| <u>References</u> | Weil, C.S., Condia, N.I. 1977. Experimental carcinogenesis of pyrolysis fuel oil. Am. Ind. Hyg. Assoc. J. 38: 730-733. |
| <u>Other</u> | |
| Last changed | 4/11/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Acute Toxicity

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|---|---|
| <u>Test Substance</u> | Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+ |
| <u>Method</u> | |
| Method/guideline followed | None specified, comparable to standard studies |
| Type (test type) | Acute |
| GLP | Not specified |
| Year | 1977 |
| Species/Strain | Rat, Wistar |
| Sex | Males and females |
| No. of animals per sex per dose | 5 |
| Vehicle | None |
| Route of administration | Oral gavage |
| Test Conditions | Male and five female Wistar rats (200-300g) were fasted for 18 hr prior to test article administration by the oral route. Test material was given by intubation at 6.67, 10.0, 15.0 and 22.5g/kg. Following intubation, rats were returned to their holding cages. Food and water were available ad lib. Rats were observed for signs of toxicity and pharmacological effects for 14 days following treatment. Data for the 15.0g/kg dose was taken from a prior project (#MB77-1855). |
| <u>Results</u> | |
| LD ₅₀ with confidence limits. | LD ₅₀ combined sexes = 14.5 (11.5-18.3)g/kg. |
| Remarks | Clinical signs were produced at all doses, but the time after dosing when symptoms were seen, was not reported. Effects at the 4 dose doses were: 6.67g/kg, diarrhea, piloerection, lethargy, ptosis; 10.0g/kg, lethargy, ataxia, tremors, coma, flaccid tone, piloerection; 15.0g/kg, lethargy, coma; and 22.5g/kg, coma, death. Rats died at 15.0g/kg (2M, 4F) and 22.5g/kg (5M, 5F). |
| <u>Conclusions</u> (study author) | LD ₅₀ combined sexes = 14.5 (11.5-18.3)g/kg. Toxicological effects were observed at all doses, but death did not occur at doses below 15.0g/kg. |
| <u>Data Quality</u> Reliability | 2. Reliable with restrictions. Observation times, when symptoms appeared, were not reported. |
| <u>References</u> | Moreno, O.M., 1977. Report on oral LD ₅₀ in rats. Project #MB77-2027, MB Research Laboratories, Inc., Spinnerstown, PA for Mobil Oil Corp, Paulsboro, NJ Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ Moreno, O.M., 1977. Report on a single dose oral toxicity study in rats. Project #MB77-1855, MB Research Laboratories, Inc., Spinnerstown, PA for Mobil Oil Corp, Paulsboro, NJ |
| <u>Other</u> Last changed | 12/18/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Acute Toxicity

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| <u>Test Substance</u> | Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+ |
| <u>Method</u> | |
| Method/guideline followed | None specified, comparable to standard studies |
| Type (test type) | Limit test |
| GLP | Not specified |
| Year | 1977 |
| Species/Strain | Rat, Wistar |
| Sex | Males and females |
| No. of animals per sex per dose | 5 |
| Vehicle | None |
| Route of administration | Oral gavage |
| Test Conditions | Five male and five female Wistar rats (200-300g) were fasted for 18 hr prior to test article administration (neat) by the oral route. Test material was given by intubation at 15g/kg. Following intubation, rats were returned to their holding cages and observed for signs of toxicity and pharmacological effects for 14 days. |
| <u>Results</u> | |
| LD ₅₀ with confidence limits. | LD ₅₀ combined sexes ~ 15g/kg (Estimated by reviewer) |
| Remarks | Two of 5 males died on days 1 and 5 (one each day) and 4 of 5 females died on day 2 (2 rats), day 3 (1 rat); day 5 (1 rat). Lethargy and coma were observed shortly after dosing. Higher mortality was seen in females |
| <u>Conclusions</u> (study author) | The combined-sex LD ₅₀ was approximately that of the 15mg/kg limit dose administered, with higher mortality seen in females. This dose is very high and approximates the maximum dose that could be placed in the rat stomach without causing aspiration. (Reviewer's comment) |
| <u>Data Quality</u> | |
| Reliability | 2. Reliable with restrictions. Very little data was provided in the report related to housing conditions and time course of clinical symptoms. GLP adherence not reported. |
| <u>References</u> | Moreno, O.M., 1977. Report on a single dose oral toxicity study in rats. Project #MB77-1855, MB Research Laboratories, Inc., Spinnerstown, PA for Mobil Oil Corp, Paulsboro, NJ Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ |
| <u>Other</u> Last changed | 12/18/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Acute Toxicity

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| <u>Test Substance</u> | Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+. |
| <u>Method</u> | |
| Method/guideline followed | None specified, comparable to standard studies |
| Type (test type) | Acute |
| GLP | Yes |
| Year | 1980 |
| Species/Strain | Rat, Sprague Dawley |
| Sex | Males and females |
| No. of animals per sex per dose | 5 |
| Vehicle | Air |
| Route of administration | Whole body Inhalation |
| Test Conditions | Five groups of Sprague Dawley rats were exposed to an aerosol of test article for a single 4 hr period at doses of 3.6±0.39, 5.2±0.08, 5.7±0.17, 8.9±0.88 and 9.3±0.79 g/m ³ ±SD. The aerosol had an equivalent aerodynamic diameter of 2.7µm±1.9 geometric SD. Rats were observed during exposure and daily thereafter for clinical signs over 14 days. Body wt was determined at initiation and at 7 and 14 days post-exposure. All rats were necropsied for gross abnormalities. |
| <u>Results</u> | |
| LC ₅₀ with confidence limits. | LC ₅₀ combined sexes ~ 6.0g/m ³ |
| Remarks | During the exposure, dyspnea, salivation, nasal discharge, and prostration were observed. Dyspnea was observed in practically all rats at all concentrations. Salivation occurred in 8 or more animals exposed to 8.9g/m ³ or greater and to a lesser degree in animals exposed to 5.7g/m ³ . Nasal discharge was observed in all groups to varying degrees. Prostration occurred in 6 or more rats given 5.7g/m ³ or greater and to a lesser degree in animals exposed to 5.2g/m ³ or less; prostration appeared to be concentration related. Males in groups exposed to 3.6 - 8.0g/m ³ weighed slightly more than pre-exposure wt at 7 days post-exposure; by 14 days post-exposure, body weight of these males was approx. normal. Females in groups given 3.6 and 5.2g/m ³ gained body wt at approx. a normal rate through 14 days post-exposure period. In the 5.7g/m ³ group females were below pre-exposure levels at 7 days post-exposure; by 14 days post-exposure, these females were at approx. normal body wt. The females in the 8.9g/m ³ group and both males and females in the 9.3g/m ³ group did not provide sufficient numbers of surviving animals to evaluate effects on body weight gain. At necropsy, a number of macroscopic abnormalities were observed but only those involving lung, liver and stomach were considered remarkable (specifics not provided). |
| <u>Conclusions</u> | |
| (study author) | LC ₅₀ combined sexes ~ 6.0g/m ³ |
| <u>Data Quality</u> | |
| Reliability | 2. Reliable with restrictions. The short report provided for review did not describe housing conditions, method of aerosol generation, and specifics on gross necropsy observations. |
| <u>References</u> | |
| | LC ₅₀ Acute inhalation toxicity evaluation in rats. 1980. IRDC, Mattawan, MI, for Mobil Chemical Co., Beaumont, TX |
| <u>Other</u> | |
| Last changed | 12/18/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Acute Toxicity

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| <u>Test Substance</u> | 140 ⁰ F vapor of Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Vapors were 30-35% alkanes/alkenes, 60-65% benzene derivatives, and 2-3% naphthalenes. Benzene, toluene, cyclopentadiene and styrene were 53% of the vapor. |
| <u>Method</u> | |
| Method/guideline followed | None specified, comparable to standard studies |
| Type (test type) | Acute |
| GLP | Yes |
| Year | 1983 |
| Species/Strain | Rat, Sprague Dawley |
| Sex | Males and females |
| No. of animals per sex per dose | 10 |
| Vehicle | Air |
| Route of administration | Whole body Inhalation |
| Test Conditions | Male and female rats (13wk old; males 349-368g, females 213-215g) were housed 5/sex/cage, in polypropylene cages with removable mesh tops and floors. Chow diet and water were available ad lib. Holding room temperature was 22±0.8 ⁰ C, relative humidity was 63±3.0%, and a 12hr light-dark cycle was maintained. Vapors were continuously generated from a supply of neat oil at 140 ⁰ F in a double-surface glass condenser. Nitrogen gas carrier was passed through the condenser, diluted with air, and metered into the exposure chambers. Chamber vapor concentrations were monitored by an infrared gas analyzer, and samples were collected every 30 min for analysis by gas chromatography/flame ionization detection. Rats received a single 4hr exposure to vapor (g/m ³ ±% variation) at analyzed concentrations of 0.59±21, 3.3±21, or 6.6±21. During exposure, rats were held in stainless steel mesh cages placed on supports in the inhalation chamber. After exposure, rats were returned to holding cages; 5 rats/sex/group were retained for a 24hr observation period, remaining 5 rats/sex/group were retained for a 14 day observation period. Rats were observed for clinical signs at 15 min intervals during exposure, 1 and 2 hrs after exposure, and once daily thereafter until sacrifice. Body wt. was recorded at initiation (day 0), on day 1, and at sacrifice; rats selected for 2wk observation were also weighed on days 2, 4, 7, and 14. Necropsies were performed on all rats. For the 24hr post-exposure sacrifice, liver, head, lungs and kidneys from all rats were weighed and processed for microscopic examination. For the 2 wk post-exposure sacrifice, lungs were weighed and processed for microscopic examination. Tissues collected from only the high-dose and control rats were examined. Final and initial body wt and organ wt were analyzed by covariance or analysis of variance, as appropriate with application of Bartlett's test. |
| <u>Results</u> | |
| LC ₅₀ with confidence limits. | LC ₅₀ was not reached at 6.6g/m ³ |
| Remarks | During exposure, visible rats showed partial eye closing and prone body posture in the 3.3 and 6.6g/m ³ groups. Slight lacrimation and abnormal breathing was noted in most rats at the high dose. During the 14-day observation period, appearance and behavior were considered normal. There were no statistically significant changes in body wt. At the 24hr post-exposure necropsy, no gross abnormalities were observed. There were no biologically significant changes in organ wt, with the exception of higher kidney wt in the 6.6g/m ³ males (p<0.05). There were no significant differences in lung wt at the 24hr or 14-day sacrifices. There were prominent intracytoplasmic eosinophilic inclusions in the renal cortical tubules of males sacrificed at 24hr. No treatment-related effects were seen in the respiratory tract at 24hr or 14-day sacrificed rats. No other significant test article-related microscopic effects were found. |
| <u>Conclusions</u> | |
| (study author) | LC ₅₀ was not reached at 6.6g/m ³ No deaths occurred during the study. Clinical signs were indicative of the presence of an irritant. The only biologically significant finding |

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| <p><u>Data Quality</u></p> <p>Reliability</p> <p><u>References</u></p> <p><u>Other</u></p> <p><i>Last changed</i></p> | <p>was microscopic eosinophilic inclusions in the renal cortical tubules of high dose males sacrificed 24hr after exposure.</p> <p>1. Reliable without restrictions.</p> <p>Rose, P.H., Jackson, G.C., Clark, G.C., and Gopinath, C. 1983. Acute inhalation toxicity in rats, 4-hour exposure. Report #MOB 9/83503. Huntingdon Research Center plc, Huntingdon, England, for Mobil Oil Corp., Princeton, NJ</p> <p>Roy, T.A. 1983. Analysis of rerun tower bottoms. Study #1271-81B,C. Mobil Oil Corp, Princeton, NJ</p> <p>12/18/2001 (Prepared by a contractor to the Olefins Panel)</p> |
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Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Acute Toxicity

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| <u>Test Substance</u> | Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+ |
| <u>Method</u> | |
| Method/guideline followed | None specified, comparable to standard studies |
| Type (test type) | Acute |
| GLP | Not specified |
| Year | 1977 |
| Species/Strain | Rabbits, New Zealand White |
| Sex | Not specified |
| No. of animals per sex per dose | 10 rabbits/dose |
| Vehicle | None |
| Route of administration | Dermal |
| Test Conditions | Ten New Zealand White rabbits were clipped free of abdominal hair and 6-10 epidermal abrasions were made longitudinally every 2-3cm over the exposed area. The abrasions were sufficiently deep to penetrate the stratum corneum but not deep enough to produce bleeding. A single 5.0g/kg dose was applied to the exposed area, which was then covered with gauze, and the trunk was wrapped with impervious material for 24 hrs. At 24 hr, the rabbits were cleansed and dermal reactions were evaluated by the Draize technique. Rabbits were then observed for 14 days. |
| <u>Results</u> | |
| LC ₅₀ with confidence limits. | One rabbit died on day 8 of the study (dosing was on day 0). All erythema and edema scores were between 1 and 2. |
| Remarks | |
| <u>Conclusions</u> (study author) | The test material was considered nontoxic by the dermal route. |
| <u>Data Quality</u> | |
| Reliability | 1. Reliable without restrictions. . |
| <u>References</u> | Moreno, O.M., 1977. Acute dermal toxicity in rabbits. Project #MB77-1855, MB Research Laboratories, Inc., Spinnerstown, PA for Mobil Oil Corp., Paulsboro, NJ Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ |
| <u>Other</u> | |
| Last changed | 12/18/2001 (Prepared by a contractor to the Olefins Panel) |

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Genetic Toxicity - in Vitro

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| <p><u>Test Substance</u> Test substance</p> | <p>Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+</p> |
| <p><u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested</p> | <p>Standard method per Ames et al, 1975 Reverse mutation bacterial assay Salmonella typhimurium with and without metabolic activation, Sacchromyces cerevisiae Yes 1977 S. typhimurium TA1535, TA1537, TA1538, TA100, TA98; S. cerevisiae D4 Yes Sprague Dawley adult male rat liver (S9 fraction) 50µl S9 fraction in 0.5ml S9 mix/plate Aroclor 1254 induced, administered to male rats 5 days prior to sacrifice 0, 0.001, 0.01, 0.10, 1.0 and 5.0µl/plate ± S9; diluted in dimethyl sulfoxide (DMSO); negative control 50 µl DMSO/plate</p> |
| <p>Statistical Methods</p> | <p>None specified. Positive response criteria per Ames et al., (1975) were number of mutant colonies equal to or greater than 2 –3 times reversion frequency of negative control for each strain. Results must be reproducible in a repeat assay.</p> |
| <p>Remarks for Test Conditions</p> | <p>Salmonella strains or S. cerevisiae D4 (approx. 10⁸/ml) were added to separate test tubes containing 2.0ml molten top agar and appropriate doses of RTB were administered to each tube. Just prior to pouring, 0.5ml S-9 mix was added to tubes receiving metabolic activation and contents was poured on minimal agar plates. Number of tubes or plates ±S9 was not reported. Plates were incubated for 48 hrs at 37⁰C and scored for number of colonies on each plate. One complete assay was performed with all strains. Repeat assays of D4 -S9, and TA1538, TA 98+S9 were performed to evaluate results from the 1st assay. Positive control compounds were: 2-nitrofluorene (NF, 100µg/plate) for TA98, TA1538 – S9; methylnitrosoguanidine (MNNG, 10µg/plate) for TA1535, TA100, D4 -S9; quinacrine mustard (QM, 10µg/plate) for TA1537 –S9 and 2-anthramine (ANTH, 100µg/plate) for TA1535, TA100 +S9, dimethylnitrosamine (DMNA, 100µM/plate) for D4 +S9, 2-acetylaminofluorene (AAF, 100µg/plate) for TA1538, TA98 +S9, and 8-aminoquinoline (AMQ, 100µg/plate) for TA1537+S9.</p> |
| <p><u>Results</u> Genotoxic effects</p> | <p>Rerun tower bottoms was toxic to Salmonella strains TA1535, TA1537, TA98 and S. cerevisiae D4 at 5.0µl/plate. Test material did not increase revertant frequency in any Salmonella strain without metabolic activation. Test of D4 was repeated at doses of 1.0 and 5.0µl/plate due to slight increased revertant frequency at 1.0µl/plate in the 1st test and toxicity at 5.0µl/plate. No increase in revertants was observed at 1.0µl and toxicity persisted at 5.0µl in the repeat assay. In the presence of S9 metabolic activation, no increase in revertant frequency was seen in TA1535, TA1537, TA100 and D4 in the first assay. A repeat test with TA98 and TA100 was performed at 1.0 and 5.0µl/plate because these strains exhibited a dose-related increase in revertant frequency [TA98+ S9: incidence of revertants was 25, 22, 33; TA1538+ S9, incidence of revertants was 26, 48, 88 at negative control, 1.0 and 5.0µl/plate, respectively]. Repeat assay was considered negative [TA98+ S9: incidence of revertants was 55, 57, 99; TA1538+ S9, incidence of revertants was 27, 44, 33 at negative control, 1.0 and 5.0µl/plate, respectively]</p> |
| <p><u>Conclusions</u> (contractor)</p> | <p>Rerun tower bottoms did not demonstrate reproducible mutagenic activity in any of the assays and was not considered to be mutagenic under these test conditions.</p> |

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| <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p> <p><u>Other</u> <i>Last changed</i></p> | <p>2. Reliable with restrictions. Report did not indicate number of tubes or plates used per dose. Criteria for a positive response were not defined in the report.</p> <p>Brusick, D.J. 1977. Mutagenicity evaluation of MCTR-36-77. LBI Proj #2683. Litton Bionetics, Inc. Kensington, MD for Mobil Chemical Co., Edison, NJ.</p> <p>Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ</p> <p>Ames, B.N., McCann, J., Yamasaki, E. 1975. Mutat. Res. 31: 347-364</p> <p>12/18/2001 (Prepared by a contractor to the Olefins Panel)</p> |
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Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Genetic Toxicity - in Vitro

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| <u>Test Substance</u> <i>Test substance</i> | Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+ |
| <u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested | Standard method, no guideline specified Sister chromatid exchange (SCE) Human lymphocytes Yes 1981 Human venous blood, primary culture No NA NA NA 1 st trial : 0, 0.0003, 0.001, 0.003, 0.011, 0.033, 0.111, 0.333, 1.1, and 3.3µl/ml 2 nd trial: 0. 0.02, 0.04, 0.06, 0.08, and 0.10µl/ml, diluted in dimethyl sulfoxide (DMSO). Negative control was 1% DMSO (100µl/culture). |
| Statistical Method | Students t-test to compare SCE frequency in treated cultures with negative controls. Positive results require approximate doubling in SCE frequency over background levels at a minimum of 3 doses. In the absence of doubling, positive response requires a statistically significant increase at a minimum of 3 doses and a positive dose response. |
| Remarks for Test Conditions | Human venous blood was drawn into a sterile heparinized syringe; cultures were initiated by adding 0.6ml blood to 9.4ml RPMI 1640 medium supplemented with fetal calf serum, 1% antibiotics and 5-bromodeoxyuridine (BrdU, 25µM), and incubated at 37°C in the dark for 24 hours. Dilutions of test material, DMSO or positive control compound, ethyl methane sulfonate (EMS, 0.1µl/ml) were added to cultures (2 cultures/ dose) and incubation continued for 46-50hrs. Colcemid was added 2-3hrs, prior to harvest, to arrest dividing lymphocytes in metaphase. After growing for 2 cell cycles in the presence of BrdU, chromosomes were fixed and stained using fluorescence (5µg/ml Hoechst 33258) with exposure to black light (15-20 min) and 5% Giemsa for an additional 10-20 min, so that one chromatid is dark and its pair or “sister” is pale, allowing exchanged segments between them to be clearly visible. Fifty cells in M2 (2 nd division metaphase)/dose were scored for frequency of SCE/cell and per chromosome. All slides were coded prior to scoring and scored “blind” |
| <u>Results</u> Genotoxic effects | In the first trial, no scorable metaphases were found at 3.3µl/ml; at 1.11µl/ml, 50 metaphases could not be found due to cell cycle delay and a reduction in mitotic index. However, there was a statistically significant increase in SCE at 1.11µl/ml and 0.33µl/ml (13.8 and 11.1 SCE/cell respectively, compared to 7.0 SCE/cell in DMSO controls). In the 2 nd trial, cultures were treated at concentrations of 0.02-0.10µl/ml and incubated for an additional 4 hours to allow cells to reach second metaphase. Pronounced cell cycle delay occurred at 0.08 and 0.10µl/ml. There were statistically significant increases in SCE/cell at 0.06 (15.1 SCE/cell), 0.08 (16.1 SCE/cell) and 0.10µl/ml (13.1 SCE/cell) compared to DMSO negative control (10.6 SCE/cell) with some evidence of a dose response. Positive control compound. EMS, induced approximately 33 SCE/cell in both assays. Doubling of SCE incidence over DMSO controls was not reached. While the SCE increase was not large, it was apparently dose related and the authors considered this effect to be a weak positive response. |
| <u>Conclusions</u> (contractor) | A small but statistically significant increase in sister chromatid exchange was demonstrated in human lymphocytes treated with RTB. Rerun tower bottoms was considered to show a weakly positive response under conditions of this assay. |
| <u>Data Quality</u> | |

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| <p><i>Reliabilities</i></p> <p><u><i>Reference</i></u></p> <p><u><i>Other</i></u></p> <p><i>Last changed</i></p> | <p>1. Reliable without restrictions</p> <p>Galloway, S.M. 1981. Mutagenicity evaluation of 081088003 in the sister chromatid exchange assay in human lymphocytes. Assay #5634. Litton Bionetics, Inc., Kensington, MD for Mobil Oil Corp, (Study # 1711-80) Princeton, NJ</p> <p>Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ</p> <p>12/18/01 (Prepared by a contractor to the Olefins Panel)</p> |
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Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Repeated Dose Toxicity

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| <u>Test Substance</u> | Rerun tower bottoms (RTB, 1271-81). Brown liquid. 140°F (60°C) vapor: 35% alkanes/alkenes, 60-65% benzene derivatives, and 2-3% naphthalenes. Benzene, toluene and styrene were 53% of vapor. |
| Remarks | |
| <u>Method</u> | |
| Method/guideline followed | Standard method, no guidelines specified. |
| Test type | Sub-acute toxicity from a Combined 10 day inhalation toxicity and teratology range-finding study |
| GLP | Yes |
| Year | 1984 |
| Species | Rat |
| Strain | Sprague Dawley [COBS:CD (SD)BR] from Charles River, UK |
| Route of administration | Whole body inhalation |
| Duration of test | 12 days |
| Doses/concentration levels | 0, 0.15, 0.74 and 5.1g/m ³ actual analyzed concentration |
| Sex | Males and females (10/sex/group) |
| Exposure period | 10 consecutive days |
| Frequency of treatment | 6 hr/day |
| Control group and treatment | 10 males, 10 females exposed to room air, 6hr/day for 10 days |
| Post exposure observation period | None |
| Statistical methods | Bartlett's test for heterogeneity of variance. Body wt., food and water consumption employed analysis of variance (ANOVA). Hematology and blood chemistry used ANOVA, William's test. For organ wts, where Bartlett's test was significant at 1%, log transformation was used to see if heterogeneity was removed. Analysis of covariance or ANOVA followed by William's test was then used for significance. |
| Test Conditions | Male and female rats (approx 13 wks old; males 240-260g, non-pregnant females 180-200g) were housed 5/sex/cage in polypropylene cages with removable mesh tops and floors, on racks within individually ventilated areas between treatment periods to prevent passive exposure to RTB vapors from rats in other dose groups. Rooms were maintained at temperature range of 20-22°C, relative humidity 60-64% and a 12 hr light/dark cycle. Rat chow and tap water were available ad lib except during exposure. Rats were assigned to groups by body weight using a pseudo-random computer program. Exposure vapors were continuously generated from a supply of neat oil at 140°F in a double-surface glass condenser. Nitrogen gas carrier was passed through the condenser, diluted with air, and metered into the exposure chambers. Generation conditions at each dose level were designed to produce approximately 20% vaporization of RTB. Total chamber airflow was set for 12 air changes/hr. Chamber vapor concentrations were monitored by an infrared gas analyzer, and samples were collected every hour on carbon sorption tubes for analysis by gas chromatography/ flame ionization detection. Target concentrations were 0, 0.1, 0.75 and 5.0g/m ³ (actual 0, 0.15, 0.74 and 5.1g/m ³) determined from an acute inhalation study. During exposure, rats were housed in stainless steel mesh cages, partitioned to isolate each rat, set on supports in the inhalation chamber. Avg. chamber atmospheres were 23.5°F, 41-59% relative humidity. Positions of animals were alternated daily. After daily exposure was completed, chambers were allowed to clear for 30 min before the rats were removed and returned to holding cages. Animals were observed twice daily for clinical signs, before and after exposure as animals were moved between cages. Animals were weighed 7 days prior to exposure and on day 1 (time 0 of exposure), 3, 7, and 10, before daily exposure began. Daily food and water consumption was recorded by cage, beginning 7 days prior to start of exposure. Blood was drawn on day 11 (the day following the final exposure) from all animals (fasted 16hr overnight). Evaluated hematology parameters were hemoglobin, red cell (RBC) count, total white cell (WBC) count and differential WBC counts, and packed cell volume (PVC); blood chemistry parameters were plasma glucose, serum total protein, serum albumin, albumin/lobulin (A/G) ratio, urea nitrogen, alkaline phosphatase, and serum glutamic-pyruvic transaminase (GPT). On day 11, all rats were killed: lungs/trachea, liver, kidneys, spleen, thymus, adrenal and gonads were weighed; 34 tissues |

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| <p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks</p> <p><u>Conclusions</u> (study authors)</p> | <p>were preserved for possible microscopic examination. Detailed microscopic examination was performed on hematoxylin-eosin stained sections of liver, kidney, testes/ovary, trachea, lung and head from all rats; thymus, spleen and adrenal from control and 5.1g/m³ groups (10M, 10F) and spleen from 10 males only in the 0.74g/m³ group.</p> <p>NOAEL was not established LOAEL both sexes = 0.15g/m³ based on statistically significant dose-related changes in kidney wt and pathology for all males, decreased urea nitrogen levels in all females (Assigned by reviewer).</p> <p>No deaths occurred during the study. Clinical signs during exposure observed in the 5.1g/m³ group were abnormal body posture, closing or partial closing of eyes on every day of exposure consistent with exposure to an irritant atmosphere. Following exposure, some 5.1g/m³ animals exhibited lethargy, red/brown staining around head or snout, urine staining, salivation, occasional ataxia and lacrimation, peripheral vasodilation mainly on the first few days of exposure, and hair loss in a few rats at the end of the 10-day treatment period. Increased urination overnight was noted in these high dose rats (both sexes). Lower dose groups showed few clinical signs. Body wt and weight gain over 10 days were similar to controls for all animals in 0.15 and 0.74g/m³ groups (male gain: 39, 40, 40 g; female gain: 12, 13, 14g in control, 0.15 and 0.74g/m³ groups, respectively. At 5.1 g/m³, males failed to gain weight (-2g), resulting in a statistically significant difference compared to controls; females gained less (8g) than controls but effect was not statistically significant. Overall, food consumption for 10 days was statistically significantly lower (p<0.01) in 5.1g/m³ males (202g) and females (149g) compared to controls (males, 248g, females, 168g), however female's food consumption became comparable to controls between days 6-9 of exposure. Other groups were similar to controls throughout. High dose rats of both sexes, particularly high dose females, consumed statistically significantly (p<0.01) more water than controls (males, 537g; females, 576g compared to control males, 324g; females 217g); all other groups were comparable to controls.</p> <p>Hematology parameters: Males exposed to 5.1g/m³ exhibited statistically significantly higher values for PCV, Hb, and RBC than controls, however the absolute values for individual animals were within historical control ranges for the laboratory; 5.1g/m³ females And other treated groups were comparable to controls.</p> <p>Blood chemistry parameters: the 5.1g/m³ males had significantly higher protein, albumin, and GPT levels and significantly lower alkaline phosphatase level than controls; in females the A/G ratio and GPT levels were higher than controls. The A/G ratio in 0.74g/m³ females was also higher than controls. Urea nitrogen levels in females at all dose levels were statistically significantly lower than controls (21, 17, 19, and 14mg/dl in control, 0.15, 0.74 and 5.1g/m³, respectively). Again, absolute values for individual rats for these parameters were within acceptable ranges for age and strain of rat. At necropsy, increased incidence of alopecia and incidence of hemorrhagic areas in the mucosa of stomachs in 5.1g/m³ female rats was observed macroscopically. Liver, kidney and adrenal wt tended to be higher than controls for most groups in a dose-related fashion; spleen and thymus wt in 0.74g/m³ and 5.1g/m³ groups tended to be less than controls. Differences were statistically significant for males at all dose levels for kidney wt, at 0.74 and 5.1g/m³ for liver (increase) and spleen (decrease), and at 5.1g/m³ only for adrenal (increase) and thymus (decrease). In females, changes were statistically significant only at 5.1g/m³. Microscopic pathology results in the 5.1g/m³ group showed enlargement of centrilobular hepatocytes in liver (5/10 males, 8/10 females), decreased cellularity of red pulp (6/10ma les) in spleen, minimal involution of thymus (3/10 males, 3/10 females), minimal increase in fine vacuolation of zona fasciculata (6/10 males, 4/10 females), and minimal increase in cortical width (7/10 females, only) in adrenals. Male rats from all dose groups has eosinophilic intracytoplasmic inclusions in renal cortical tubules in the kidney. Other changes at 5.1g/m³ or in other dose groups were considered spontaneous in origin and of no toxicological significance.</p> <p>NOAEL was not established. Decreases in body wt gain and food intake, increases in water intake, changes in hematology, blood chemistry and organ wts, and pathological findings were induced by rerun tower bottoms in rats exposed for 10 days. RTB appeared to induce diuretic action with compensatory water intake in both sexes. Incomplete compensation in male rats resulted in minimal hemoconcentration (increased PCV, Hb, RBC and total protein</p> |
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| <p><u>Quality</u> Reliabilities</p> <p><u>References</u></p> <p><u>Other</u> Last changed</p> | <p>at 5.1g/m³); in female rats, a decrease in urea nitrogen (all doses) resulting from increased urine flow. Direct effect on the liver was demonstrated by elevated GPT levels, and enlargement of centrilobular hepatocytes in both sexes at the high dose. The occurrence of intracytoplasmic inclusions in renal cortical tubules was observed in male rats in all groups, increasing in severity with dose. [This effect could later be correlated with characteristic hydrocarbon-induced nephropathy seen in male rats. Reviewer's comment]. Changes in adrenals, spleen and thymus probably correlate with stress response and adaptation to exposure to Rerun tower bottoms.</p> <p>1. Reliable without restrictions</p> <p>Rose, P.H., Street, A.E., James, P. and Gopinath, C. 1984. Compound 1271-81 vapor ten-day inhalation toxicity and teratology range-finding study in rats. HLS-MOB7/83656. Huntingdon Research Centre, plc. Cambridgeshire, England for Mobil Oil Corp, Princeton, NJ (see separate summary for teratology data).</p> <p>Rose, P.H., Jackson, G.C., Clark, G.C., and Gopinath, C. 1983. Acute inhalation toxicity in rats, 4-hour exposure. Report #MOB 9/83503. Huntingdon Research Center plc, Huntingdon, England, for Mobil Oil Corp., Princeton, NJ</p> <p>Roy, T.A. 1983. Analysis of rerun tower bottoms. Study #1271-81B,C. Mobil Oil Corp, Princeton, NJ</p> <p>12/18/01 (Prepared by contractor to Olefins Panel)</p> |
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Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Developmental Toxicity/Teratogenicity

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| <u>Test Substance</u> | Rerun tower bottoms (RTB, 1271-81). Brown liquid. 140°F (60°C) vapor: 35% alkanes/alkenes, 60-65% benzene derivatives, and 2-3% naphthalenes. Benzene, toluene and styrene were 53% of vapor. |
| Remarks | |
| <u>Method</u> | |
| Method/guideline followed | Standard method, no guidelines specified. |
| Test type | Teratology range-finding from a Combined 10 day inhalation toxicity and teratology range-finding study |
| GLP | Yes |
| Year | 1984 |
| Species | Rat |
| Strain | Sprague Dawley [COBS:CD (SD)BR] from Charles River, UK |
| Route of administration | Whole body inhalation |
| Concentration levels | 0, 0.15, 0.74 and 5.1g/m ³ actual analyzed concentration |
| Sex | Time-mated females (5/ group) |
| Exposure period | Day 6-15 of gestation |
| Frequency of treatment | 6 hr/day |
| Control group and treatment | 5 time mated females, exposed to room air 6hr/day from day 6-15 of gestation |
| Duration of test | 20 days |
| Statistical methods | None performed. Small sample size in range-finding study |
| Remarks for Test Conditions. | <p>Twenty sexually mature time mated female rats (171-200g at study initiation) were assigned to one of four groups (5/group) based on body wt on day 1 of gestation. The day of mating identified by presence of a vaginal plug =day 0 of gestation. Rats were housed 5/group in polypropylene cages with stainless steel mesh floors and tops when not in exposure chambers. Animal rooms were maintained at 20-22°C temperature, 60-64% relative humidity and a 12hr light/dark cycle. Rat chow and tap water were available ad lib except during exposure. Exposure vapors were continuously generated from a supply of neat oil at 140°F in a double-surface glass condenser. Nitrogen gas carrier was passed through the condenser, diluted with air, and metered into the exposure chambers. Generation conditions at each dose level were designed to produce approximately 20% vaporization of RTB. Total chamber airflow was set for 12-air changes/hr. Chamber vapor concentrations were monitored by an infrared gas analyzer, and samples were collected every hour on carbon sorption tubes for analysis by gas chromatography/ flame ionization detection. Target concentrations were 0, 0.1, 0.75 and 5.0g/m³ (actual 0, 0.15, 0.74 and 5.1g/m³) determined from an acute inhalation study. During exposure, rats were housed in stainless steel mesh cages, partitioned to isolate each rat, set on supports in the inhalation chamber. Avg. chamber atmospheres were 23.5°F, 41-59% relative humidity. Positions of animals were alternated daily. After daily exposure was completed, chambers were allowed to clear for 30 min before the rats were removed and returned to holding cages. Animals were observed twice daily for clinical signs, before and after exposure, and from post-treatment days 16-20 of gestation. Rats were weighed on day 1, 3, 6, 10, 14, 17 and 20 of gestation. Food and water consumption were recorded daily by cage. On day 20 of gestation, rats were killed by CO₂ asphyxiation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. Ovaries and uteri were examined for number of corpora lutea, number and distribution of live fetuses, embryonic/fetal death, fetal abnormalities and individual live fetal wt from which litter wt was calculated. Uteri or individual uterine horns without visible implantations were immersed in 10% ammonium sulfide to evaluate pregnancy. Live fetuses were examined externally and weighed. One half of fetuses in each litter were preserved in Bouin's fixative for soft tissue evaluation by free-hand sectioning if required, one half were fixed, sexed internally, eviscerated, cleared and stained with Alizarin Red for skeletal examination if required. Group mean values for litter size, embryonic death, and pre- and post-implantation loss were calculated.</p> |
| <u>Results</u> | |
| NOAEL maternal toxicity | NOAELmaternal = 0.74g/m ³ (Assigned by reviewer) |

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| NOAEL developmental toxicity | NOAEL developmental = 0.15g/ m ³ (Assigned by reviewer) |
| Maternal effects | During exposure, 5.1g/m ³ females only showed closing or partial closing of eyes, inactivity and abnormal body posture. Between exposures, 5.1g/m ³ females showed lethargy, red staining of snout, slight general vasodilation, and increased urination and staining of urogenital region with occasional signs of slight ataxia and increased salivation. At 0.74g/m ³ , one rat had red staining on snout on 2 occasions. At 5.1g/m ³ , food consumption was reduced during the treatment period and water consumption was markedly increased during and post-treatment; other groups were similar to controls. At 5.1g/m ³ , body wt loss occurred during the first four days of treatment (-1.0g compared to 21.2, 25.0 and 19.3g in control 0.15, and 0.74g/m ³ groups, respectively); body wt gain in the 0.74g/m ³ group was marginally lower than controls, but regained parity by day 14. From days 14-20 of gestation, high dose weight gain improved but did not reach parity with other dose groups. At necropsy, no gross abnormalities were observed in parental animals. Pregnancy rate was 80-100% in all groups, incidence of corpora lutea, implantations, and live young were comparable to or higher in treated groups than controls. |
| Embryo/fetal effects | At 5.1g/m ³ , mean fetal wt was markedly reduced (2.97g compared to 3.57g in controls) and mean number of intra-uterine deaths was higher (1.6 compared to 0.6 in controls); these effects of lesser magnitude were also observed in the 0.74g/m ³ group litters. No fetal malformations were observed at necropsy. |
| <u>Conclusions</u> (study authors) | Rerun tower bottoms induced clinical signs of exposure in pregnant rats, principally at the highest dose level, 5.1g/m ³ , and marginal body wt loss and decreased food consumption during the early days of exposure; increased water consumption was observed throughout exposure and the post-treatment period. Mean fetal wt was reduced and mean number of intra-uterine deaths was increased at 5.1g/m ³ . No teratogenic effects (malformations or variations) were observed in offspring at necropsy. The doses employed in this assay would be acceptable for a definitive teratology study of Rerun tower bottoms. |
| <u>Data Quality</u> <i>Reliabilities</i> | 1. Reliable without restrictions |
| <u>References</u> | Rose, P.H., Street, A.E., James, P. and Gopinath, C. 1984. Compound 1271-81 vapor ten-day inhalation toxicity and teratology range-finding study in rats. HLS -MOB7/83656. Huntingdon Research Centre, plc. Cambridgeshire, England for Mobil Oil Corp, Princeton, NJ (see separate summary for toxicity data). Rose, P.H., Jackson, G.C., Clark, G.C., and Gopinath, C. 1983. Acute inhalation toxicity in rats, 4-hour exposure. Report #MOB 9/83503. Huntingdon Research Center plc, Huntingdon, England, for Mobil Oil Corp., Princeton, NJ Roy, T.A. 1983. Analysis of rerun tower bottoms. Study #1271-81B,C. Mobil Oil Corp, Princeton, NJ |
| <u>Other</u> <i>Last changed</i> | 12/18/01 (Prepared by contractor to Olefins Panel) |

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Developmental Toxicity/Teratogenicity

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| <u>Test Substance</u> | Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+ |
| Remarks | |
| <u>Method</u> | |
| Method/guideline followed | Standard method, no guidelines specified. |
| Test type | Teratology study |
| GLP | Yes |
| Year | 1981 |
| Species | Rabbit |
| Strain | New Zealand White |
| Route of administration | Dermal |
| Concentration levels | 0, 62.5, 125, 250, 500 and 1000mg/kg/day |
| Sex | Females (5/ group) |
| Exposure period | Day 6-18 of gestation |
| Frequency of treatment | Once daily |
| Control group and treatment | 5 females/group, distilled water applied dermally from day 6-18of gestation |
| Duration of test | 29 days |
| Statistical methods | None performed. Small sample size in range-finding study |
| Remarks for Test Conditions. | <p>Thirty sexually mature, virgin female rabbits (8.5-9.5 months old at time of insemination), had been acclimated for 30 days, ear-tagged with a unique number for identification, and assigned by body wt to one of 5 groups (5 females/group). All rabbits were individually housed in suspended wire mesh cages and maintained in a temperature and humidity controlled room (specifics not given) with a 12hr light/dark cycle. Rabbit chow and tap water was available ad lib. During the treatment period, all animals were maintained in a specially ventilated room under identical housing conditions. Females were artificially inseminated with semen from one of 3 proven male rabbits of same strain and source. Semen was collected using an artificial vagina; only semen with 50% or greater motility was used. Ejaculate was diluted with 4.0ml of 0.9% NaCl, USP injectable grade at 35°C and 0.25-0.5ml was introduced into the anterior vagina. Within one hour after insemination, ovulation was induced by injection of 100units of chorionic gonadotropin into the marginal ear vein. Day of insemination =day 0 of gestation. Semen from one male was used to inseminate an equal number of females in each group. Prior to test article administration (gestation day 6) and as often as necessary thereafter, the back of each female was shaved and each animal was fitted with a Saf-T Shield® collar. Rerun tower bottoms was applied undiluted to the intrascapular area in a single daily application on gestations days 6-18. The site was uncovered, non-abraded and was not washed. Control rabbits were treated with distilled water at a concentration of 1.08ml/kg in a comparable regimen. Dosages were determined from individual body wt at day 6; collars were removed on gestations day 19. Prior to, during treatment, and until day 29 of gestation, females were observed for mortality, overt changes in appearance and behavior, and clinical signs of toxicity (day 6-29 of gestation). Females showing signs of abortion remained on study until scheduled sacrifice to determine any test article related effect; aborted tissue was discarded. Gross necropsy was performed on all rabbits not surviving to scheduled necropsy. Maternal body wt were recorded on gestation days 0, 6, 12, 18, 24 and 29. Dams were sacrificed on day 29, uteri were excised and the location of viable and non-viable fetuses, early and late resorptions, number of total implantations and corpora lutea were recorded. Abdominal and thoracic cavities and organs of dams were examined for gross evidence of morphological changes and discarded. Uteri from apparent non-gravid females were opened and placed in 10% ammonium sulfide solution for confirmation of pregnancy status.</p> |
| <u>Results</u> | |
| NOAEL maternal toxicity | NOAEL maternal was not established (Rabbits at lowest dose aborted) |
| NOAEL developmental toxicity | NOAEL developmental was not established. (Reviewer's comments). |

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| Maternal effects | <p>All animals in control, 62.5, 125 and 250mg/kg groups survived to the end of the study. One 500mg/kg female aborted and died on gestation day 229, and 3 died in the 1000mg/kg day group – one on day 21, 2 on day 27 of gestation (1 aborted prior to death). Prior to death, matting and/or staining of hair in the urogenital region and lack of coordination of the hind limbs was noted. At necropsy, reddened foci and/or focal erosion of stomach lining was observed, suggesting that, despite collars, inherent preening activity and subsequent oral ingestion of RTB occurred. Two females that died in the 1000mg/kg group had tan, creamy intestinal contents; distention of gall bladder was also noted in one of these rabbits. In the 1000mg/kg rabbit that died on day 21 of gestation, the left uterine horn was 1/3 as long as the right horn with thickening of the endometrium. Eleven rabbits aborted: 2 each in control, 62.5 and 500mg/kg/day groups, 3 in 125mg/kg group and 1 each in the 250 and 1000mg/kg groups. Since 40% (2/5) control rabbits aborted, inhalation of RTB may be a contributing factor. Dermal irritation at application site, erythema, edema, fissuring, was observed in all treated rabbits, first reported on day 13 of gestation and persisting until sacrifice. Eschar formation was reported later in the treatment period (days not given) and persisted until sacrifice. Nasal discharge, soft stool, occasional instances of dry, yellow matter in nasal region, hair loss, and white ocular discharge were noted in controls and treated animals with similar frequency. At necropsy, one female in the 62.5mg/kg group had a 2cm diameter abscess on the ventral neck; one 125mg/kg female had several erosions in the lining of the gall bladder, and one female had slight pitting of kidneys; one 500mg/kg female had accentuated lobulation of the liver. Fluid in the abdominal cavity was observed in one 62.5mg/kg female and one 500mg/kg female. Mean maternal body wt losses occurred in all treated groups during the treatment period (day 6-18 of gestation). Mean body wt gains were seen following the end of exposure (day 18-29 of gestation) in the 62.5, 125 and 500mg/kg groups; mean gains exceeded controls. Continued wt loss occurred in the 250 and 1000mg/kg groups post-treatment. Post-implantation loss occurred in the control group and in treated groups below or within laboratory historical control values (0.4-1.6); losses did not occur in a dose-related pattern. No biologically meaningful differences were observed in mean number of corpora lutea, total implantations or viable fetuses in any treatment group compared to controls. Decrease in mean number of total implants correspond to decrease in mean number of viable fetuses in 125 (one dam), 250 and 500mg/kg/day groups- one dam in each group had only early resorptions. Mean number of total implants and viable fetuses in 1000mg/kg group was greater than control mean values (9.5 implants and 8.5 fetuses in 1000mg/kg group compared to 6.7 implants and 6.7 fetuses in controls). No grossly observed teratogenic effects were reported.</p> |
| Embryo/fetal effects | |
| <p><u>Conclusions</u> (study authors)</p> | <p>Dermal irritation, erythema, edema, fissuring and eschar formation, were induced by Rerun tower bottoms in all treated groups. Mean maternal body wt losses occurred during treatment (day 6-18 of gestation). Four rabbits died (1 in 500mg/kg, 4 in 1000mg/kg group); 2 of which aborted prior to death. Eleven rabbits aborted, including 2 in control group, suggesting that inhalation of RTB may have been a contributing factor. Due to high abortion rate in every treatment group, a dose of 62.5mg/kg/day would be excessive for definitive teratology study.</p> |
| <p><u>Data Quality</u> <i>Reliabilities</i></p> | <p>1. Reliable without restrictions</p> |
| <p><u>References</u></p> | <p>Spicer, E.J.F and Schardein, J.L. 1981. Pilot dermal teratology study in rabbits (MCTR-169-79). IRDC Proj. #450-013. International Research and Development Corp., Mattawan, Mich. for Mobil Oil Corp., Princeton, NJ Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ</p> |
| <p><u>Other</u> Last changed</p> | <p>12/18/01 (Prepared by contractor to Olefins Panel)</p> |

Robust Summary: Heavy Fuel Oil Rerun Tower Bottoms

Developmental Toxicity/Teratogenicity

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| <u>Test Substance</u> | Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+ |
| Remarks | |
| <u>Method</u> | |
| Method/guideline followed | Standard method, no guidelines specified. |
| Test type | Teratology study |
| GLP | Yes |
| Year | 1981 |
| Species | Rabbit |
| Strain | New Zealand White |
| Route of administration | Dermal |
| Concentration levels | 0, 10, 25, and 50mg/kg/day |
| Sex | Females (16/ group) |
| Exposure period | Day 6-18 of gestation |
| Frequency of treatment | Once daily |
| Control group and treatment | Proximate and remote control groups: 16 females/group, distilled water applied dermally from day 6-18 of gestation |
| Duration of test | 29 days |
| Statistical methods | All analyses compared proximate to remote control and each control group to each treatment group ($p < 0.05$). Chi square with Yates correction and/or Fisher's exact probability test used for male to female sex distribution and number of litters with malformations. Mann-Whitney U-test used for number of early and late resorptions and post-implantation losses. One-way analysis of variance, Bartlett's test, appropriate t-test for equal or unequal variance and Dunnett's multiple comparison tables were used for other fetal parameters and mean fetal body wt. |
| Remarks for Test Conditions. | Eighty sexually mature, virgin female rabbits (approx. 7 months old at time of insemination), had been acclimated for 63-68 days, ear-tagged with a unique number for identification, and assigned by body wt to one of 3 groups (16 females/group). All rabbits were individually housed in suspended wire mesh cages and maintained in a temperature and humidity controlled room (specifics not given) with a 12hr light/dark cycle. Rabbit chow and tap water was available ad lib. During the treatment period, all animals were maintained in a specially ventilated room under identical housing conditions. Remote control females were housed separately. Females were artificially inseminated with semen from one of 10 proven male rabbits of same strain and source. Semen was collected using an artificial vagina; only semen with 50% or greater motility was used. Ejaculate was diluted with 4.0ml of 0.9% NaCl, USP injectable grade at 35°C and 0.25-0.5ml was introduced into the anterior vagina. Within one hour after insemination, ovulation was induced by injection of 100units of chorionic gonadotropin into the marginal ear vein. Day of insemination = day 0 of gestation. Procedures were performed on 2 consecutive days and equal numbers of females were inseminated in each group/day. Prior to test article administration (gestation day 6) and as often as necessary thereafter, the back of each female was shaved and each animal was fitted with a Saf-T Shield® collar. Doses were selected following a pilot range-finding study. Rerun tower bottoms was applied undiluted to the intrascapular area in a single daily application on gestations days 6-18. The site was uncovered, non-abraded and was not washed. Control rabbits were treated with distilled water in a comparable regimen. Dosages were determined from individual body wt at day 6; collars were removed on gestations day 19-20. Prior to, during treatment, and until day 29 of gestation, females were observed for mortality, overt changes in appearance and behavior, and clinical signs of toxicity (day 6-29 of gestation). Females showing signs of abortion were sacrificed and examined for grossly evident morphological changes; intact fetuses were examined externally and preserved in formalin. Gross necropsy was performed on all rabbits not surviving to scheduled necropsy, and tissue was saved in formalin, if appropriate. Maternal body wt were recorded on gestation days 0, 6, 12, 18, 24 and 29. Due to a weighing error on |

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| | <p>gestation day 18, body wt from 8 females in the 25mg/kg group were not included in calculations of group mean. Dams were sacrificed on day 29, uteri were excised and weighed prior to removal of fetuses. The location of viable and non-viable fetuses, early and late resorptions, number of total implantations and corpora lutea were recorded. Abdominal and thoracic cavities and organs of dams were examined for gross evidence of morphological changes and discarded. Uteri from apparent non-gravid females were opened and placed in 10% ammonium sulfide solution for confirmation of pregnancy status. All fetuses were individually weighed and examined for external malformations and variations. Each was internally sexed and examined for visceral malformations and variations including brain by mid-coronal slice, and heart dissection by modification of Staples' technique. Eviscerated skinned fetuses were individually numbered and tagged, fixed in alcohol, macerated in KOH and stained with Alizarin red S for skeletal examination.</p> |
| <u>Results</u> | |
| NOAEL maternal toxicity | NOAEL maternal was not established (Dermal irritation at all levels) |
| NOAEL developmental toxicity | NOAEL developmental = 50mg/kg/day (Levels assigned by reviewer). |
| Maternal effects | <p>All animals in control, 10, and 25mg/kg groups survived to the end of the study. One 50mg/kg non-gravid female died on gestation day 11, exhibiting edematous lungs and hyperemic tracheal mucosa at necropsy, suggesting respiratory distress as the cause of death. There were 14/16 apparently gravid rabbits in the proximate control, 10/16 in the remote control, 14/16, 12/16 and 14/16 in the 10, 25 and 50mg/kg groups, respectively. Eight rabbits aborted and were sacrificed; one each in proximate control (gestation day 23) and 25 mg/kg group (gestation day 26), 2 in 10mg/kg group (gestation day 26), and 4 in 50mg/kg group (One each on gestation day 20, 22, 27, 28). Majority of rabbits that aborted in the 50mg/kg group had congested lungs or foci on lungs at necropsy. Dermal irritation at application site was observed in all treatment groups. Redness and swelling of application site was evident at initiation of treatment and persisted throughout gestation. Majority of females developed fissuring and flaking midway through treatment that persisted until sacrifice. Peeling of epidermal layer was reported in 7 rabbits, one at 10, 2 at 25 and 4 at 50mg/kg groups for several days during the treatment period. Dermal irritation did not occur in either control group. Soft stool was evident in the majority of rabbits treated with 50mg/kg/day. No treatment related findings were observed in organs of maternal females at necropsy. There were no biologically meaningful differences in mean maternal body wt gain between controls, or between any treated group and remote controls during the treatment period (day 6-18 of gestation)- gains were 147, 212, 145 and 148 in remote control, 10, 25 and 50mg/kg/day groups, respectively. Comparable losses in mean adjusted body wt (female body wt on gestation day 29 minus uterus and contents) were observed in both controls and all treated groups [-104, -272, -55, -171, -244 in proximate controls, remote controls, 10, 25 and 50 mg/kg/day, respectively. Since a substantial adjusted body wt loss was observed in remote control animals, these changes were not considered treatment- related.</p> |
| Embryo/fetal effects | <p>At caesarean section, there were no statistically significant or biologically meaningful differences in mean number of corpora lutea, total implantations, post-implantation loss, early or late resorptions, viable fetuses or fetal sex distribution between controls or in 10, 25 or 50mg/kg/day groups. The incidence of gravid dams with viable fetuses was 100% in all groups: 13, 10, 12, 11, and 10 and viable fetuses/dam were 6.7, 6.7, 5.6, 7.1, and 7.0 in proximate control, remote control, 10, 25, and 50mg/kg/day groups. A decrease in mean number of corpora lutea. A decrease in mean number of corpora lutea and total implantation with corresponding decrease in mean number of viable fetuses was observed in the 10mg/kg day group when compared to remote controls, but effect were not observed at higher doses and were not considered treatment related. A lower mean fetal body wt. was observed at the 50mg/kg/day level (36.4g compared to 40.4g in proximate control and 42.9g in remote controls) but was within the laboratory's historic control range (35.2-42.6g). There were no biologically meaningful or statistically significant differences in number of litters with malformations in either control group or RTB-treated groups. Hydrocephaly was observed in 4 fetuses in one litter in the 10mg/kg group and 3 fetuses in 2 litters (2 in one litter and 1 in one litter) in the 25mg/kg/day group. No hydrocephaly was seen at 50mg/kg/day so these findings were considered not treatment related. Spina bifida occurred in one fetus in one 50mg/kg/day group litter. An increase</p> |

